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THE EFFECT OF STILBESTROL ON EXPERIMENTAL STREPTOCOCCAL INFECTION IN MICE[1]

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EARLY MEDICAL literature is replete with explanations of the causation of disease derived from long observation of the individual and his response to external environment. Lacking the tools of modern biological science, these early students by necessity devoted considerable attention to the idea of variations of the host in his reaction to a supposedly constant environment. Joseph Gallup, writing in Vermont in 1815, stated:

"This state of the human body has been denominated a predisposition to disease; but we are not here to understand it as being actual disease; only a susceptibility or aptitude to particular diseased action in the system, when thrown into any state of commotion by exciting causes, which are very numerous. . . . It may be a very curious question to determine, what actual change takes place in the system, that should render it so liable to the attack of fever from slight causes, which would not be noticed at another time. . . . If these positions be true, the too much neglected science of physiology—not only the physiology of nature, but the physiology of experience and observation, appears, in the majesty of its dignity, to assert its station in the first rank of medical requirements."

The discovery of bacteria and the evolution of the germ theory diverted the center of attention from the host to the micro-organism —with the new tools of bacteriological science, lending itself more readily to investigation. Such concentration of interest has resulted in exquisite differentiations of bacteria; and although adding greatly to our knowledge by pointing out experimentally many differences among strains of the same species, has not yet furnished an adequate explanation for the phenomenon of disease. It becomes evident that

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type differentiation does not in itself constitute the disease producing quality when it is considered that different types of the same species will cause the same disease; often types known to be disease producers in some do not do so in others.

With refinements of bacteriological technique, there has been an increasing trend towards the laboratory study of disease. This has brought about the disappearance of the "horse and buggy" practitioner with his knowledge accumulated through long association in his practice with variations in the reactions of individuals or entire families to disease. None the less, in the experimental approach to the study of disease, clear-cut differences in host susceptibility often have been encountered; indeed, the use of particular strains of experimental animals to ensure uniform results has become a laboratory commonplace. Perhaps the reason for the slowness to readily include these laboratory implications in epidemiologic concepts has been the lack of proper tools for precise studies of the human host with respect to variable reactions to a given infectious agent.

Notable among the laboratory demonstrations of host variation are the genetic variations in susceptibility to experimental cancer, (Bauer, 1932), Webster's experiments with inbred stocks of widely different susceptibilities to the same infectious agent (Webster 1934), and the work on tissue permeability and "spreading factors" (Duran-Reynals, 1942).

As is well known, pregnancy affects the development or progress of several diseases. These effects may be the result of mechanical or nutritional stresses, or may result from some intrinsic physiologic changes; probably related to the economy of endocrine hormones during pregnancy. Certain endocrine disorders are brought about or accentuated by pregnancy: toxic goiter may be accentuated, and pituitarism may be influenced. On the other hand, there is no adequate explanation for some of the effects noted; notable among those lacking sufficient explanation are the "therapeutic effect" of pregnancy on syphilis, and the resistance of pregnant animals to experimental syphilitic infection. It is thought by some that pregnancy may be a predisponent to lethargic encephalitis (MacNalty, 1926, Alpers and Palmer, 1929), and to paralytic poliomyelitis (Aycock, 1941). However, the exact nature of these physiologic differences still is not clearly understood. As Gallup (1815) stated:

> "The very delicate functions of the human system, as connected with nervous irritability, are intricate and difficult to be understood; and more especially to be expressed, from the poverty of language. In these particulars, physiology acknowledges its ignorance."

A number of studies in recent years are beginning to draw attention to the importance of such factors as heredity, nutrition and endocrine function in the susceptibility and resistance to disease. The

therapeutic effect of estrogenic hormone in juvenile vaginitis (Burpee, Robinson and Leslie, 1939), the alteration of susceptibility to experimental poliomyelitis induced by estrogens (Aycock, 1936–1937) and the role of subclinical endocrinopathy in susceptibility to human poliomyelitis (Aycock, 1940) suggest that the economy of estrogenic hormones may be one factor in the autarceology of infection.

During the course of studies on the apparent association of Lancefield Group D streptococci to severe, often fatal infections in premature infants and young children, it became apparent that the distribution of particular serological types of the micro-organism did not explain the occurrence disease (Wheeler and Foley). Repeated attempts to demonstrate experimentally some evidence of pathogenicity for these streptococci were unsuccessful. These data, together with the relative infrequency with which these micro-organisms were encountered in infections among the older age-groups suggested that host factors in those exposed might be the determinant of disease. In view of the limitation of the infections, and the effect of estrogenic substances on certain other experimental infections, the effect of these substances on experimental streptococcal infection with Group D and other streptococci was studied in mice. Because of an idea that had been advanced suggesting that the absence of Vitamin K might be involved in the apparent increased susceptibility of young children (Rubenstein), Vitamin K in corn oil[2] as well as stilbestrol in peanut oil[3] was used in the first series of test animals.

Four strains of Group D streptococci of different serological types (Wheeler and Foley) were selected for the preliminary experiments. Mouse virulence was low-grade, 0.25 ml–0.5 ml of a 12–18 hour broth culture being required to kill an animal 15–22 gm in weight within 48 hours. Serial mouse passage in the presence of mucin (Nungester, 1932) enhanced virulence slightly, enabling a dose of 0.25 ml to kill regularly within 48 hours. Forty-eight mice, 24 male and 24 female, 15–22 gm in weight, were given a single injection of 40–50 R.U.[4] of stilbestrol subcutaneously. Forty-eight hours later, these mice were given 0.5 ml of a 12–18 hour broth culture intraperitoneally. An equal number of male and female controls were given 0.25 ml of the same culture intraperitoneally; 6 of the stilboestrol-treated group and 6 normal control mice were injected with each of the four strains selected.

In addition to the normal control mice injected with each strain of streptococci, a series of mice injected subcutaneously with 0.25 ml of sterile peanut oil were included as a control on the stilbestrol solvent. Since the eight mice which were given thyloquinone in corn

[2] 2-Methyl-1, 4-Naphthoquinone in corn oil, E. R. Squibb & Sons.
[3] Stilbestrol in peanut oil, E. R. Squibb & Sons.
[4] Equivalent to 400–500 I.U.

oil subcutaneously 48 hours previous to intraperitoneal injection of the same cultures showed no resistance, these mice can be considered as additional controls.

In the mice receiving a preliminary injection of stilbestrol, the mortality rate following inoculation with culture was 14.3% as compared with a mortality rate of 80.9% in the other groups of mice, which received one-half the dose of culture used in the stilboestrol-treated mice (table 1). There was no significant difference between the mortality of the normal control and oil control mice.

In a second experiment designed to confirm the results recorded in table 1, and to determine the duration of stilbestrol-induced re-

TABLE 1. EFFECT OF STILBESTROL ON EXPERIMENTAL GROUP D STREPTOCOCCUS INFECTION IN MICE

Mice	No.	Sex	Treatment	Dose of culture	Survived*	Died	Mortality
Stilbestrol	24	male	40–50 I.U. stilbestrol in peanut oil	0.5 ml	20	4	16.1
	24	female			22	2	9.1
Total	48				42	6	14.3
CONTROLS							
Vitamin K	4	male	0.1 mg thyloquinone in corn oil	0.25 ml	1	3	75.0
	4	female			0	4	100.0
Oil	6	male	0.25 ml sterile peanut oil**	0.25 ml	1	5	83.3
	6	female			2	4	66.6
Normal	24	male	none	0.25 ml	6	18	75.0
	24	female			3	21	87.6
Total	68				13	55	80.9

* Survival time 48 hours.

** Represents approximately twice the volume of oil used as vehicle for 40–50 R.U. of stilbestrol.

sistance, a highly virulent culture of hemolytic streptococci (Dochez N.Y.-5, Lancefield Group A, Griffith Type 10)[5] was used. This strain has been maintained in mice for several years; doses of 0.0001–0.001 ml killing a mouse 18–22 gm in weight within 48 hours.

A group of two hundred mice was divided into two lots of one hundred each, with equal numbers of male and female mice in each lot. One lot was treated with a single subcutaneous injection of 40–50 R.U. of stilbestrol. After 48-hours, and at 48-hour intervals thereafter, groups of 5 male, 5 female stilbestrol-treated mice and an

[5] This strain was received through the kindness of Miss J. M. Coffey, Division of Laboratories and Research, New York State Department of Health.

equal number of male and female control (normal) mice were injected intraperitoneally with 0.001 ml of a 12–18-hour broth culture of hemolytic streptococci.[6] On the fourteenth day of the experiment, the stilbestrol-treated mice having become as "normal controls" (figure 1), the remaining control mice were given 40–50 R.U. of stilbestrol subcutaneously, thereby reversing the experiment.

As shown in figure 1, in the group of stilbestrol-treated mice injected with hemolytic streptococci, up to the eighth day following administration of the stilbestrol, the mortality was markedly re-

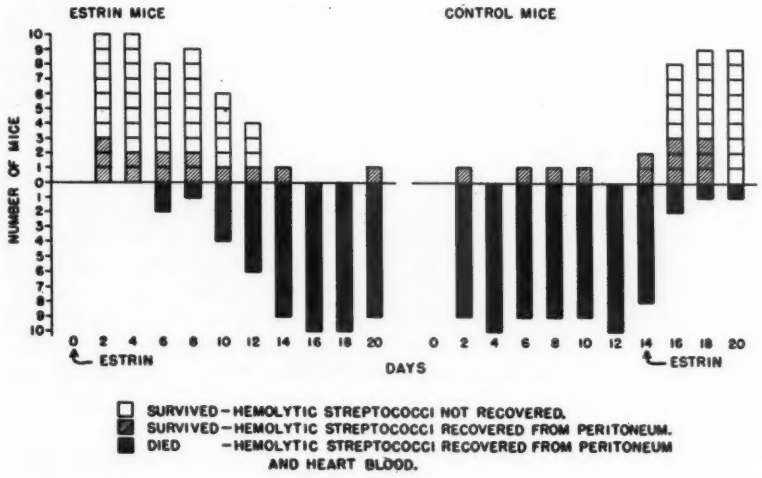

FIG. 1. Effect of estrin (stilbestrol) on experimental group A streptococcus. Infection in mice: Duration of protection and post mortem bacteriology.

duced. Between the eighth and tenth days the mortality in the stilbestrol-treated mice approached fifty per cent—still considerably less than the mortality in the control mice.

All mice were autopsied; those which survived 48 hours following injection of the culture were killed and autopsied. Heart blood and peritoneal fluid were cultured in beef infusion broth and on five per cent horse blood agar. As is shown in figure 1, hemolytic streptococci were recovered from the heart blood and peritoneum of the majority of control mice, while only occasionally from the stilbestrol-treated mice; positive heart blood cultures appearing only after the fifth day following injection of the stilbestrol.

Von Haam and Rosenfeld (1942) reported on the degree of protection afforded against experimental pneumococcus infection in mice by graded doses of various sex hormones. The present study

[6] Diluted with sterile broth so that a volume of 0.5 ml contained 0.001 ml of culture.

was designed to test maximal protection against maximal mortality; no attempt was made to determine the degree of protection afforded against experimental streptococcal infection in mice by graded doses of stilbestrol. The mechanism by which these substances alter the resistance of the host to various infectious agents is not clearly understood. Bactericidal and bacteriostatic activity is not exhibited by the natural estrogens (Faulkner, 1943). The failure of stilbestrol-treated monkeys to develop immunity to poliomyelitis following intranasal instillation of the virus (Aycock, 1936–1937), and the work of Sprunt (1941) with vaccinia virus suggest that protection is afforded by physiologic changes induced in the tissues which affect the role of the "spreading factors" in infection. On the other hand, the increased antibody response in vaccinated animals receiving estrus-inducing substances suggests that protection in the hormone-treated animals lies in an enhanced antibody production (von Haam, 1942, von Haam and Rosenfeld, 1942).

Postmortem bacteriological examination of the stilboestrol-treated and control mice in the present experiment suggests that the administration of stilbestrol interferes with the spread of hemolytic streptococci in mice (figure 1). Furthermore, stilbestrol rendered mice resistant to infection without previous experience with the micro-organisms. There is some data on the optimal time relationship between administration of estrogenic substance and exposure to the micro-organism (von Haam and Rosenfeld, 1942). These data, together with the results of the present experiments, do not indicate the intrinsic lag between exposure to the infectious agent and the development of antibodies, as is characteristic of most antigen-antibody relationships.

SUMMARY

A single dose of 40–50 R.U. of stilbestrol rendered mice highly resistant to a dose of mouse virulent hemolytic streptococci which regularly killed normal control mice of the same age and sex. This protective action was of eight to ten days duration. The results of postmortem bacteriological examination suggest that protection was afforded by some stilboestrol-induced factor which interfered with the spread of hemolytic streptococci in mice.

This experiment is another example which illustrates the importance of host rather than parasitic variation in determining disease upon exposure to the infectious agent.

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THE PITUITARY RESPONSES OF MATURE MALE RATS TO AN OXIDATIVE INACTIVATION PRODUCT OF ESTRONE[1]

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ALTHOUGH a number of investigators have been concerned with the problem of the metabolism of estrogens and the mechanism of the inactivation process which takes place within the body, few compounds known to be degradation products of naturally occurring estrogens have been identified. In 1942 Westerfeld reported the isolation and identification of a lactone recovered after the *in vitro* oxidation of crystalline estrone with hydrogen peroxide. Our interest in this compound stems from the fact that throughout our studies on the urinary excretion of estrogens by women we have been impressed by an apparent correlation between the clinical conditions of the patients and the pathway of estrogen metabolism. As a result of our observations the theory has been advanced that estrogen degradation products may have certain properties which have been attributed to estrogens *per se*. Before describing our experiments with Westerfeld's lactone, a brief summary of the findings which have led to this concept is in order.

It has been found that the estrogenic potency of the urine may be greatly increased over that present after the optimum conditions of simple acid hydrolysis by subjecting the urine to HCl in the presence of an excess of zinc dust (Smith and Smith, 1937 and 1941). All of the evidence to date, albeit circumstantial, favors the conclusion that most, if not all, of the activity of urine after Zn-HCl treatment which cannot be accounted for by estrogens excreted as such, *i.e.*, "unaccounted-for T_{zn} activity," is derived from inactivation products of secreted or administered estrogens produced *in vivo*. The most convincing evidence in support of this conclusion is that, after estrone administration to women, there is an increase in "unaccounted-for T_{zn} activity" amounting to 10 to 60 per cent of the estrogen given (Smith, Smith and Schiller, 1942 (a) and unpublished data). The urinary excretion of this apparently non-estrogenic material which may be rendered estrogenic by Zn-HCl treatment differs under varying conditions, being least when the partition of urinary estrogens

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[1] The Mrs. William Lowell Putnam Investigation of the Toxemias of Pregnancy.

indicates a maximum rate of estradiol to estrone to estriol conversion, and greatest when total estrogens are low and the rate of conversion is much reduced. The former situation has been found to be most pronounced in women at times when progesterone secretion is at its height, while the latter, which is most marked when progesterone is deficient, may be reversed by administration of progesterone, testosterone or adrenal cortical extract in adequate dosage.[2] It has been concluded, therefore, that the rate of formation of inactivation products of estrogens which may be rendered estrogenic by Zn-HCl hydrolysis is greatest at times when estrogens are being secreted or administered in the absence of an adequate production of the protective steroids.

Correlation of the data collected from women under various physiological, pathological and experimental conditions has led to the concept that inactivation products may have certain properties attributed to the estrogens but exhibited by them only indirectly when protective steroids are deficient and inactivation rather than conversion predominates. This concept would be considerably strengthened by the demonstration that some estrogenically inactive compound, known to be a product of estrogen, exhibited those physiological effects which our studies have led us to believe may be accountable to estrogen metabolites rather than to estrogens *per se*. It was for this reason that we were interested in investigating the physiological properties of Westerfeld's lactone. Thus far only one of the biological responses ordinarily attributed to estrogens *per se* has been studied, namely, the stimulation and release of gonadotropic hormones of the anterior pituitary.

Before investigating the pituitary responses to Westerfeld's lactone, we were interested in determining whether or not its estrogenic activity could be increased by Zn-HCl treatment. Our assay of the untreated lactone in 0.005 N NaOH, *i.e.*, the open ring form, using the vaginal response of standardized mature spayed rats, shows it to have one-fifteenth of the estrogenic activity of estrone. This agrees very well with Westerfeld's assay of the open

[2] Evidence that progesterone increases the rate of estrogen excretion was first presented by Smith and Smith in 1931, working with rabbits, and in 1932, in women. This was later confirmed and elaborated upon by Pincus and Zahl (1937) and by Pincus and Graubard (1940). Subsequent investigations in this laboratory have consistently indicated that in women, as was first shown by Pincus and Zahl in rabbits, estradiol to estrone to estriol conversion is enhanced when the proper balance of progesterone and the estrogens pertains and depressed, with an increase in the rate of estrogen destruction, when progesterone is deficient. Heard, Bauld, and Hoffman (1941), working with rabbits and using strictly chemical methods, failed to substantiate this finding, but these investigators administered very large doses of α estradiol (300 mg.), and the same amount of progesterone. We have found that, in women, at least 5 times as much progesterone as estrogen must be injected in order to influence estrogen excretion to any marked extent. That testosterone and adrenal cortical extract have an effect similar to that of progesterone upon estrogen metabolism has been indicated by analyses for urinary estrogens in women after administration of these substances (Smith and Smith, 1940; Smith, Smith and Schiller, 1941; and Smith, Smith and Gauld, 1943).

ring form on spayed mice. In the closed ring form, however, *i.e.*, a very dilute solution of the lactone in phosphate buffer at pH 6.6, the activity is greatly diminished, showing only one-sixtieth of that of estrone. Treatment with zinc and HCl has been found to give a 5-fold increase in potency over that of the open ring form and a 20-fold increase over that of the true lactone. Westerfeld was unable to effect reduction of the lactone acetate by treatment with metallic sodium. In our experiments a very dilute solution of the lactone (100 to 150γ in 700 cc. of a concentrated buffer solution containing 69 gm. of NaH_2PO_4 and 179 gm. of Na_2HPO_4 per liter) was subjected to 3 hours of refluxing after the addition of 5% zinc and 15 volumes per cent of concentrated HCl. This solution was then extracted either 4 times with 200 cc. of ether or for 24 hours in a continuous benzene extractor and the organic solvent evaporated down into olive oil for biological assay. Whether or not reduction of the lactone was accomplished by this procedure remains to be determined. The fact that its estrogenic potency was greatly increased and that the urine of women contains some non-estrogenic material which is rendered estrogenic by the same treatment supplies suggestive evidence in favor of the possibility that the lactone or some similar compound may be excreted as a metabolite of the estrogens. The attempts of Heard and Hoffman (1941) to find it in the urine of a man after massive estrogen administration were unsuccessful, and these authors concluded that the major part of the hormone was inactivated through an 'oxidative attack in the ortho or para position with respect to the phenolic hydroxyl group.' Their evidence, however, is admittedly entirely negative and the possibility that rupture of ring D at the carbonyl group, such as was accomplished by Westerfeld in producing the lactone, is also an *in vivo* route of estrogen inactivation is still open to investigation. It seems quite likely that more than one pathway of destruction may be followed and that the formation of estrogenically inactive compounds which may be rendered estrogenic by Zn-HCl treatment may ordinarily be of minor significance from a quantitative point of view. Our studies of 'unaccounted-for T_{ea}' activity after estrogen administration have, in the majority of cases, accounted for only a small percentage of the 'lost' estrogen. Partial degradation, on the other hand, which may well be what we are dealing with, might be of major significance from a physiological standpoint.

EXPERIMENTAL

It is well established that the enlargement of the ovaries and adrenals of rats which follows the short-time administration of estrogens is mediated by the stimulation of hypophyseal gonadotropic and adrenotropic factors. A decrease in the transplantable gonadotropic activity of the pituitary has also been found to follow treatment with estrogens, but this apparent contradiction is explained by the fact that estrogens cause release of gonadotropic hormone as well as increased secretion, so that the gland itself, although secreting more, contains less (Severinghaus, 1936; and Wolfe and Brown, 1942). The experiments to be reported were aimed towards determining whether or not these particular responses to estrogens in mature male rats could be elicited by Westerfeld's lactone. Control experiments with

estrone were also run in order to be certain that any effect of the lactone was not referable to its slight estrogenic activity. The influence of protective steroids upon the reactions to the lactone and to estrone, respectively, was also studied.

PROCEDURE

The weight of the adrenals and pituitary and the transplantable gonadotropic activity of the pituitary were measured. Sexually mature (10 to 13 weeks old) male rats, weighing between 140 and 200 gm., were used. In preliminary experiments large variations were found in uninjected controls, even though animals of the same weight were compared. When littermate was compared with littermate, however, these variations were greatly reduced, provided rats of the same weight (within 5 gm.) were used and the differences expressed in percentage figures. For each injected animal, therefore, a control littermate was simultaneously autopsied and the difference between experimental and control organ weights expressed in percentage increase or decrease. In the experiments with castrated animals, operation was performed upon the control littermate at the same time as upon the animal to whom injections were to be given, the injections being started the next day. The material to be administered was freshly made up in 0.005 N NaOH and injected twice daily for 1 to 12 days. The rats were killed 40 hours after the last injection when the period of administration was 1 to 3 days, and 16 hours after the last injection when the 5- or 12-day injection period was followed. Each experiment was run upon 2 animals at a time with 2 littermate controls, so that 2 pituitaries would be available for transplantation. The pituitaries, after being weighed, were ground in saline and injected over 3 days into 21-day-old female rats. The ovaries of these recipients were weighed on the fifth day, and the weight of the ovaries of an animal that had received 2 pituitaries from injected donors compared with that of a littermate of the same weight that had received 2 pituitaries from control donors, the difference again being expressed as percentage increase or decrease.

RESULTS

The findings are presented in the accompanying tables. Twenty-four control experiments on intact rats (table 1-A) and 14 on castrated animals (table 3-A) were run, in which the procedure was exactly the same throughout as when estrone or the lactone was given except that 0.005 N NaOH alone was injected to one of the 2 littermates. From the mean differences observed in these control experiments and the standard deviations, the significance of the experimental results upon castrated and intact animals, respectively, may be judged.

In 20 experiments on intact males, 12.6 to 117γ of the lactone[3]

[3] Two different samples of the lactone were used in this investigation. The first, an aliquot of the material originally analyzed and identified by Dr. Westerfeld (melting point 335° C.) was generously provided by him. The second, through the interest of Dr. Gregory Pincus of Clark University, Worcester, Mass., was kindly donated by Dr. A. Mather, who was working with this compound from a different angle. This sample was not analyzed, but was prepared according to the method described by Westerfeld (1942). Eighteen mgm. of lactone acetate, which had been recrystallized to a con-

TABLE 1. ADRENAL AND PITUITARY WEIGHTS AND TRANSPLANTABLE GONADOTROPIC ACTIVITY OF PITUITARIES OF INTACT MATURE MALE RATS

Injections into one of 2 intact littermates			Change in weight vs. uninjected littermate		Pituitary recipients (21-day female rats)
	Total dose (γ)	No. of days	Adrenals %	Pituitary %	Change in weight of ovaries[1] %
A. Effect of the Lactone					
The Lactone (Westerfeld[2])	12.6	1	+ 5.7	− 3.7	−27.8
	12.6	1	+ 7.5	− 2.8	
	12.6	5	+14.5	+ 2.8	−50.7
	12.6	5	+16.0	+ 4.2	
	27.0	5	+29.2	+ 1.8	−28.2
	27.0	5	+35.0	− 3.2	
	54.0	3	+36.4	+ 5.1	−52.0
	54.0	3	+33.5	+ 3.8	
	54.0	5	+50.0	+21.2	−32.0
	54.0	5	+38.2	+25.5	
	117.0	12	+26.5	+15.0	−27.5
	117.0	12	+45.5	+13.4	
The Lactone (Mather[2])	53.0	5	+14.0	+14.2	−26.1
	53.0	5	+25.0	+19.4	
	53.0	5	+25.5	+24.0	−21.8
	53.0	5	+27.5	+42.0	
	53.0	5	+19.2	+21.5	−21.3
	53.0	5	+32.9	+26.2	
	53.0	5	+26.5	+29.5	−23.1
	53.0	5	+13.0	+32.1	
24 control expe'ts with 0.005 N NaOH only		3–5	+2.2 ± 4.3	+0.7 ± 4.3	+0.7 ± 5.7
B. Effect of Estrone					
Estrone	10.0	3	+6.0	−1.9	−3.3
	10.0	3	−1.6	−0.5	
	10.0	5	−0.7	0	+6.3
	10.0	5	+7.7	+3.9	
	50.0	3	+5.9	+4.5	−7.0
	50.0	3	+9.3	+1.5	
	50.0	5	+2.6	+2.2	+0.8
	50.0	5	+0.8	+3.9	
	50.0	5	−9.5	+4.3	+2.5
	50.0	5	+2.1	−2.0	
	120.0	12	−2.7	+6.7	−7.0
	120.0	12	+3.2	+1.7	

[1] Versus littermate that received 2 pituitaries from uninjected control intact donors.

[2] See Footnote 3, page 149.

stant melting point of 142° C., were hydrolyzed to give 13 mgm. of the lactone (browning at 305°C. and melting with decomposition at 309°C.). Dr. Mather's sample, assayed in dilute NaOH, had a slightly higher estrogenic potency than Dr. Westerfeld's (*i.e.*, one-tenth that of estrone rather than one-fifteenth), and showed somewhat less increase after Zn-HCl treatment (*i.e.*, a 3-fold rather than a 5-fold increase over the open ring form). As seen in tables 1-a and 4-c, however, it elicited the same physiological responses in mature male rats. Another sample provided by Dr. Mather had no demonstrable estrogenic activity either before or after Zn-HCl treatment and had no

over 1 to 12 days resulted, in every experiment (table 1-A), in a significant decrease in the transplantable gonadotropic activity of the pituitary. It is interesting that 117γ over 12 days had no greater influence in this respect than a single injection of 12.6γ. Cytological studies have provided convincing evidence that the decreased gonadotropic potency of the pituitaries of estrogen-treated rats is accountable to release of hormone rather than decreased secretion (Severinghaus, 1936; and Wolfe and Brown, 1942). The above observation indicates that the same mechanism applies to the action of the lactone, since, if the pituitary were depressed, one would expect a cumulative effect with more prolonged administration.

The smallest amount of the lactone which gave pronounced en-

TABLE 2. EFFECT OF THE LACTONE UPON THE ADRENALS OF HYPOPHYSECTOMIZED MATURE MALE RATS

Injections to one of 2 hypophysectomized littermates		Change in weight of adrenals vs. uninjected littermate
Total dose (γ)	No. of days	%
54	5	− 7.8
54	5	− 5.4
90	5	− 6.1
90	5	−10.3

largement of the adrenals was 27γ, while 54γ were required to cause a significant increase in the weight of the pituitary and then only when injections were spread over 5 rather than 3 days. In other words, it would appear that release of gonadotropic hormone is the immediate effect of very small amounts of the lactone, whereas increased secretion of adrenotropic hormone follows larger dosages, and even larger amounts over a longer period are required to cause hypertrophy of the pituitary.

The estrogenic potency of the lactone used in these experiments was equivalent to that of 0.85 to 7.8γ of estrone. From the completely negative results in 12 experiments on intact males given 10 to 120γ of estrone over 3 to 12 days (table 1-B), it is apparent that the slight estrogenic activity of the lactone is in no way responsible for the pituitary responses observed.

In order to be certain that the adrenal enlargement following injections of the lactone was mediated by the pituitary, as has been shown to be the case with estrogens (Selye and Collip, 1936), 4 experiments were run using 8 hypophysectomized rats (table 2). Fifty-four

effect on the pituitaries of rats. This sample also was not analyzed but had a melting point which more closely approached that of Dr. Westerfeld's, *i.e.*, browning at 325°C. and melting with decomposition at 330 to 340°C. Attempts in this laboratory to produce the lactone, following Dr. Westerfeld's procedure exactly, have been unsuccessful.

to 90γ of the lactone were injected to one of 2 littermates during 5 days starting the day after operation. There was no resultant hypertrophy of the adrenals, although 54γ over 5 days was consistently effective in intact animals.

In 4 experiments on castrated rats (table 3-A), 54 to 117γ of the lactone over 5 to 12 days caused a definite enlargement of the adrenals and pituitaries and a decrease in the transplantable activity of the

TABLE 3. ADRENAL AND PITUITARY WEIGHTS AND TRANSPLANTABLE GONADOTROPIC ACTIVITY OF THE PITUITARIES OF CASTRATED MATURE MALE RATS

Injections to one of 2 castrated littermates			Change in weight vs. uninjected littermate		Pituitary recipients (21-day female rats) Change in weight of ovaries[1]
	Total dose (γ)	No. of days	Adrenals %	Pituitary %	%
A. Effect of the Lactone alone					
The Lactone (Westerfeld[2])	54	5	+30.0	+18.3	−38.0
	54	5	+40.3	+33.3	
	117	12	+38.5	+12.8	−42.0
	117	12	+45.0	+37.0	
14 control experiments with 0.005 N NaOH only		3–5	+3.7 ± 5.1	−0.9 ± 5.9	+0.3 ± 3.6
B. Effect of Estrone alone					
Estrone	10	5	+19.8	+ 4.3	−41.2
	10	5	+15.9	+ 9.2	
	30	5	+24.5	+15.0	−53.7
	30	5	+22.7	+12.8	
	50	5	+25.5	+28.1	−40.0
	50	5	+25.0	+47.0	
	50	5	+38.0	+29.0	−39.0
	50	5	+31.0	+31.0	
	120	12	+50.0	+27.0	−45.5
	120	12	+30.5	+27.2	

[1] Versus littermate that received 2 pituitaries from uninjected control castrated donors.
[2] See Footnote 3.

pituitaries. The degree of response did not differ significantly from that of intact animals to the same dosages, indicating that the presence of the testes has no influence upon these reactions to the lactone. This is in marked contrast to the results with estrone. In 10 experiments upon castrated rats (table 3-B), the same dosages of estrone which were completely ineffective upon intact animals resulted, in every case, in a significant increase in the weight of the adrenals and decrease in the transplantable activity of the pituitary. Significant enlargement of the pituitary was also observed in all except the group of rats that received less than 50γ of estrone.

It has been shown by numerous lines of investigation that castration results in an increase in the gonadotropic activity of the pituitary, FSH production being predominantly affected. There is also general agreement that castration of male rats is followed by adrenal and pituitary hypertrophy (Tepperman *et al.*, 1943). The changes observed after injections to castrated males must have been over and above those of castration, since the control littermates were simultaneously operated upon, but it might be argued that castration rendered the pituitaries more responsive to estrone administration. The fact that the responses to the lactone, however, were uninfluenced by castration suggests that this could not entirely explain the marked difference between castrated and intact animals. A logical interpretation is that the lactone exerts its effects through direct stimulation of the pituitary, whereas estrone acts indirectly through the formation of the lactone or some similar compound whose production is inhibited by testicular secretion. Such an interpretation is in keeping with the finding that testosterone has an effect similar to that of progesterone in reducing the rate of estrogen degradation in women (Smith and Smith, 1940).

The comparative influence of progesterone upon the responses of castrated rats to the lactone and to estrone was next investigated. Although there is considerable evidence for direct inhibition of the pituitary by progesterone, comparatively large doses are required for this effect (Cutuly, 1941; and Astwood and Fevold, 1939). If the stimulation and release of pituitary gonadotropic and adrenotropic factors which follows small doses of estrone is accountable to inactivation products rather than to estrone *per se*, inhibition of this response should result from very much less progesterone (which depresses the rate of estrogen degradation) than is required for any direct inhibition of the pituitary.

Six mature male rats (table 4-A), with a littermate control for each, were castrated and given 167γ of progesterone in 0.1 cc. of olive oil. During the following 5 days a total of 50γ of estrone and 833γ of progesterone were injected in divided doses to the experimental animals. There was no significant enlargement of the adrenals or pituitaries or decrease in the transplantable activity of the pituitaries. This is in striking contrast to the changes that followed injections of 50γ of estrone alone to castrated males. In 4 similar experiments (table 4-B) the same amount of progesterone alone gave no evidence of direct pituitary inhibition. In 6 experiments (table 4-C), following exactly the same procedure, it was found that the effect of 53γ of the lactone was entirely uninfluenced by the simultaneous administration of 1 mg. of progesterone. Again one is led to believe that the lactone acts directly on the pituitary, whereas estrone has its effect through some intermediary compound similar to the lactone, whose formation is depressed by the presence of progesterone.

According to the results of urinary recovery experiments in women (Smith, Smith and Schiller, 1942 (b)) and in men (Schiller and Pincus, 1943), estriol is very much less readily destroyed by the animal organism than the other naturally occurring estrogens. Six castrated mature male rats, with an uninjected littermate for each (table 5), were given 100 to 200γ of estriol in 10 injections over 5 days starting

TABLE 4. ADRENAL AND PITUITARY WEIGHTS AND TRANSPLANTABLE GONADOTROPIC ACTIVITY OF THE PITUITARIES OF CASTRATED MATURE MALE RATS

Injections to one of 2 castrated littermates			Change in weight vs. uninjected littermate		Pituitary recipients (21-day female rats) Change in weight of ovaries[2]
	Total dose (γ)	No. of days	Adrenals %	Pituitary %	%
A. Effect of Estrone plus Progesterone[1]					
Estrone	50	5	+ 2.4	− 7.1	−1.5
	50	5	+11.2	− 1.6	
	50	5	+ 7.8	+ 3.3	−1.7
	50	5	− 2.6	− 8.3	
	50	5	− 1.2	+ 1.9	−5.7
	50	5	+ 5.3	+ 6.1	
B. Effect of Progesterone alone					
	0	5	+ 3.9	+ 6.2	− 2.0
	0	5	+ 8.3	+ 6.2	
	0	5	− 1.6	+ 4.5	+ 2.5
	0	5	− 6.1	0	
C. Effect of the Lactone plus Progesterone					
The Lactone (Mather[3])	53	5	+32.0	+42.8	−28.2
	53	5	+38.0	+25.6	
	53	5	+20.3	+19.6	−37.5
	53	5	+20.5	+13.5	
	53	5	+27.0	+24.2	−25.0
	53	5	+20.0	+21.0	

[1] 1000γ in divided doses to the experimental littermate donor, starting the day of castration.

[2] Versus littermate that received 2 pituitaries from uninjected control castrated donors.

[3] See Footnote 3.

the day after castration. There was no significant enlargement of the pituitaries or adrenals, and the transplantable activity of the pituitaries did not differ significantly from that of the control donors. This is in contrast to the effect of estrone in castrated rats, in which one-tenth as much hormone caused definite changes.

DISCUSSION

From the consistency of the results presented it seems safe to conclude that Westerfeld's lactone, a known product of estrone inactiva-

tion, does exhibit certain pituitary stimulating properties which have been attributed to the estrogens. The findings demonstrate that these properties are specific for the lactone and not related to its slight estrogenic activity. Furthermore, the observation that castration or progesterone administration has no influence upon the responsiveness of mature male rats to the lactone, whereas the presence of the gonads or the administration of progesterone (both of which would depress the rate of estrogen inactivation) completely nullifies the responses to an amount of estrone which is otherwise effective, strengthens the hypothesis that the release and stimulation of pituitary gonadotropic

TABLE 5. ADRENAL AND PITUITARY WEIGHTS AND TRANSPLANTABLE GONADOTROPIC ACTIVITY OF PITUITARIES OF CASTRATED MATURE MALE RATS

Effect of Estriol				
Injections into one of 2 castrated littermates		Change in weight vs. uninjected littermate		Pituitary recipients (21-day female rats) Change in weight of ovaries[1]
Total dose (γ)	No. of days	Adrenals %	Pituitary %	%
100	5	−0.4	+1.7	−3.3
100	5	+1.7	+3.1	
100	5	+4.1	+5.9	+6.3
100	5	−9.6	−3.1	
200	5	−2.1	+1.2	−6.7
200	5	+4.7	−6.1	

[1] Versus littermate that received 2 pituitaries from uninjected control castrated donors.

and adrenotropic factors which result from estrone administration are not direct estrogenic effects, but are accountable to some intermediary product in the metabolic inactivation of estrone, such as the lactone.

It should be emphasized that there is as yet no direct evidence that such a compound is a physiological product of estrogen metabolism. The possibility, however, is still open to investigation. Experiments aimed towards fractionating human urine in such a manner as to localize and finally identify the precursor of 'unaccounted-for T_{zn}' activity are underway. If the increased potency of human urine which may be produced by Zn-HCl hydrolysis is derived from some partial degradation product of the estrogens similar to Westerfeld's lactone, then our studies in women indicate that this route of inactivation ordinarily accounts for only a small percentage of the estrogenic potency which cannot be recovered after estrogen administration. Such partial degradation products, however, may be of major physiological significance. The fact that Westerfeld's lactone is physiologically active in very small dosages, much smaller than the amount of estrone required for similar effects, is in keeping with this idea.

The question arises as to whether or not Westerfeld's lactone or some similar compound could be formed in the oxidative inactivation

of estrogens other than estrone and known to exert the same pituitary stimulating effects. A number of investigators have reported adrenal and pituitary enlargement following the administration of α estradiol and diethylstilbestrol. A compound similar to the lactone as a metabolite of α estradiol is quite conceivable, since conversion of α estradiol to estrone *in vivo* has been demonstrated (Heard and Hoffman, 1941). Such a substance as a product of the inactivation of diethylstilbestrol, however, is impossible, considering the marked difference between the chemical structure of this and the naturally occurring estrogens. It is not inconceivable, however, that partial degradation products of diethylstilbestrol,[4] although of a structure quite different from that of the lactone, might have similar biological properties. After all, why should diethylstilbestrol be estrogenic?

Considerable further investigation, both from a chemical and biological aspect, of this lactone and of other compounds that might arise from estrogen inactivation is indicated. Are such compounds concerned in the capillary permeability and edema of the endometrium associated with an increase in the rate of estrogen degradation at the time of menstruation, in cervical metaplasia, cornification of the vaginal mucosa or edema of the sex skin of monkeys? All of these reactions to the estrogens have been inhibited by progesterone or testosterone in adequate dosage (Hisaw, 1943). We can already say with some assurance that vaginal cornification cannot be explained on this basis, since Westerfeld's lactone is so much less effective than estrone in this respect. The modification of this and certain other reactions to the estrogens by progesterone or testosterone, as, for example, the development of the lobule-alveolar system of the mammary glands and the proliferation of the endometrium, would appear to be direct rather than through any effect upon estrogen metabolism. From the results presented, however, the concept is considerably strengthened that some of the physiological effects which have been attributed to the estrogens may actually be accountable to products of oxidative inactivation and are exhibited by the estrogens only indirectly when protective steroids are deficient and inactivation rather than conversion predominates.

SUMMARY

Experiments with Westerfeld's lactone, an oxidative inactivation product of estrone, have revealed that this compound causes, in much smaller dosages, the same release and stimulation of the gonadotropic and adrenotropic factors of the anterior pituitary of mature male rats as estrone itself. Neither castration nor progesterone administration

[4] In this connection it is worthy of note that diethylstilbestrol administration to a castrate woman resulted in an increase in 'unaccounted-for T_m' activity equivalent to approximately 10 per cent of the estrogen given, while the recovery of estrogens excreted as such was only 2.5 per cent (Smith and Smith, unpublished data).

influences the pituitary responses to the lactone, whereas the effect of corresponding amounts of estrone are completely nullified by the presence of the testes or by progesterone administration (both of which depress the rate of estrogen inactivation). It is concluded that estrone exerts these effects indirectly, through formation of the lactone or some similar compound.

These findings are in keeping with the previously advanced hypothesis, derived from studies of estrogen metabolism in women, that certain of the physiological effects ordinarily attributed to estrogens *per se* are actually accountable to estrogen breakdown products and are exhibited by the estrogens only indirectly when protective steroids are deficient and the pathway of metabolism is predominantly one of inactivation rather than conversion.

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THYROID AND METATHYROID DIABETES

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IN PREVIOUS work under my direction, the influence of the thyroid on pancreatic diabetes and on the endocrine function of the pancreas has been studied from the physiological and pharmacological points of view. This paper is a continuation of those studies.

METHODS AND TECHNIQUE

Experiments were made in dogs to study: 1) The effect of thyroidectomy on diabetes; 2) the diabetogenic action of thyroid treatment; 3) the characteristics of thyroid and metathyroid diabetes.

The majority of the animals used were males of 7 to 12 kg. weight, fed a daily ration of 35 to 45 gm. beef, 100 ml. milk and 10 gm. sucrose, water *ad. lib.* The urinary volume was measured daily, as also the blood sugar (Hagedorn & Jensen) and glycosuria (Benedict); nitrogen (Kjeldahl) and ketone bodies were determined in the urine of 5 days (Van Slyke). Bovine thyroid powder was given daily by stomach tube; it had 0.36% iodine, 3% ash, 2.1% humidity. Experiments were made only on animals in good general condition and after the operative wounds had healed completely.

Total thyroidectomy was performed taking care to leave the parathyroid glands; if their circulation seemed unsatisfactory they were grafted into the neighboring muscles. During the week following the operation the dogs were fed a diet of milk and bread, and were given 5 to 10 gm. calcium gluconate by stomach tube; afterwards they were fed the usual meat, milk and sugar diet and the calcium gluconate was discontinued. Pancreatectomy was usually subtotal, 2.5 to 3 gm. of gland were left around Santorini's duct and close to the duodenum. During the 5 to 7 days following the operation, the animals were injected with 5 to 10 units of insulin daily, so that the fragment of pancreas remaining could recover.

ACTION OF THYROID IN NORMAL DOGS

The series of 25 dogs studied with De Finis has been enlarged to a total of 76, of which 29 have been used by Brignone.[1] These animals with intact pancreas), received 0.5, 1.2 and 4 gm. per kg. daily of thyroid powder for 6 to 16 days, only 3 were given 0.5 gm. per kg. daily during 1 month. Those treated with 2 and 4 gm. had a higher mortaility specially during the summer.

Blood sugar increased in many animals, mostly among those receiving 1 to 4 gm.; there were great variations, but the highest figure

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[1] Brignone will publish a thesis with detailed facts on the changes in blood sugar found in these animals.

recorded was 149 mg.%. Diabetes was not observed in any of these animals, neither in the course of, nor following treatment.[2] Microscopical examination of the pancreas was made in 12 cases; 5 had no alterations, 5 showed a decrease in the granules of some β cells and 2 showed slight vacuolization in a few and loss of granules in many β cells.

The pancreas taken from 8 dogs treated for 15 days with 0.5 gm. per kg. per day were grafted into the blood vessels of the neck of diabetic dogs, pancreatectomized 24 hours before. A normal pancreas in these conditions lowers the blood sugar from 200–250 mg.% to levels below

TABLE 1. TIME IN HOURS NECESSARY FOR RECOVERY OF NORMAL BLOOD SUGAR LEVEL (120 MG.% OR LESS) IN A PANCREATECTOMIZED DOG 24 HOURS AFTER OPERATION, WITH HYPERGLYCEMIA (200 TO 250 MG. %), AFTER GRAFTING A PANCREAS INTO THE BLOOD VESSELS OF THE NECK

	No. of cases returning to normal blood sugar after hours				
Pancreas	3	4	5	6[1]	6[2]
Normal	5	5	5	—	2[3]
Dogs fed thyroid: 0.5 gm. per kg. daily for 15 days	—	1	2	3	2

[1] A fall in blood sugar not returning to 120 mg.%.
[2] No decrease or an insignificant decrease in blood sugar.
[3] One had obesity and a fatty liver.

120 mg.% in 3 to 5 hours. The pancreas from a thyroid treated animal usually required 5 to 6 hours to do this, an indication of a diminished capacity of insulin secretion in an emergency, such as the need to correct a diabetic hyperglycemia (table 1).

DIABETOGENIC ACTION OF THYROID IN DOGS WITH A REDUCED PANCREAS

Subtotal pancreatectomy was performed in dogs weighing 7 to 12 kg. (preferably 7 to 8.5 kg.); from 2.5 to 3 gm. of gland were left around the major pancreatic duct close to the duodenum. Only in 3 cases 4 gm. were left. This operation was performed because previous work, done since 1932, has shown that anterohypophyseal diabetes can be the more easily obtained, the smaller the amount of pancreas there is.

The blood sugar was examined for long periods (15 to 385 days) and in those animals in which a normal level was constantly maintained, thyroid treatment by stomach tube was commenced. The initial dose was 0.5 gm. per kg. daily; in resistant animals it was increased to 1 g. and in some to 2 gm. per kg. daily. Results depend on the dose, the duration of the treatment and the previous history of the animals.

[2] Similar negative results have been observed in white rats with daily doses of 5, 10, 25, 50 and 100 mg. of thyroid powder, given over periods lasting from several weeks to months.

Sometimes a transitory diabetic condition, with a rapid return to normal blood sugar, was observed; in other cases a permanent diabetes was obtained. Thyroid diabetes is the name given here to diabetes observed during thyroid treatment; metathyroid diabetes is that persisting definitely after discontinuing treatment. This last is

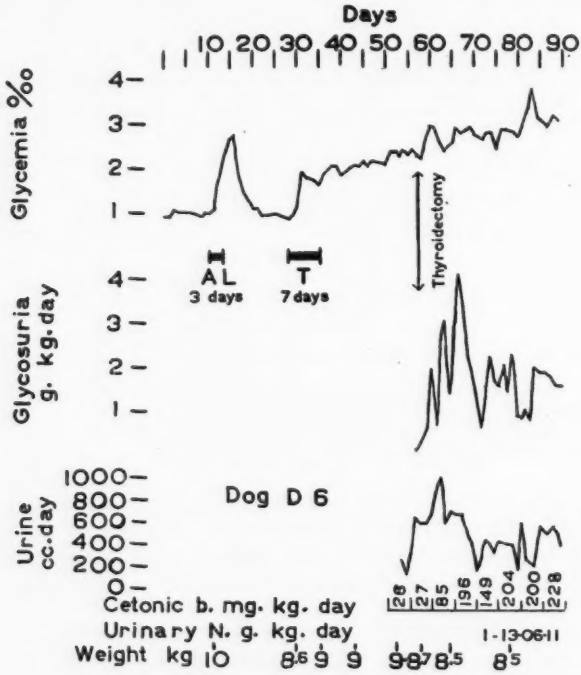

FIG. 1. Permanent metathyroid diabetes provoked by ingestion of powdered bovine thyroid (0.5 gm. per kg. daily for 7 days) in a dog with pancreas reduced to $\frac{1}{8}$ of its weight. Blood sugar had remained normal for 201 days following subtotal pancreatectomy.

AL anterior hypophyseal extract. T thyroid feeding. Thyroidectomy was performed when in metathyroid diabetes.

due to insufficiency of the islets, it is therefore a pancreatic diabetes produced by thyroid treatment, but not maintained by it.

Recently operated dogs seem to be more sensitive, since a metathyroid diabetes was obtained by giving 0.5 gm. per kg. daily during 15 to 25 days (Dogs H 1, H 2, D 11, H 10, H 11, H 8). In cases with a long standing operation it was necessary to repeat this treatment (Dogs DF 7, D 8) or to increase the dose (Dogs H 6, H 5, H 4, H 3) in order to produce permanent diabetes.

Animals were sensitized by a previous thyroid treatment and they reacted to a second treatment with a more marked hyperglycemia, and a permanent diabetes. Injection of anterior hypophyseal extract,

producing a transitory diabetes, also sensitized animals to the diabetogenic action of the thyroid (Dogs DF 7, D 6, D 8, H 9, H 6a, H 7) (Figs. 1 and 2).

A metathyroid diabetes was obtained in 15 dogs.[3] It was not obtained in 7 animals, 2 of which had 4 gm. pancreas remaining and were

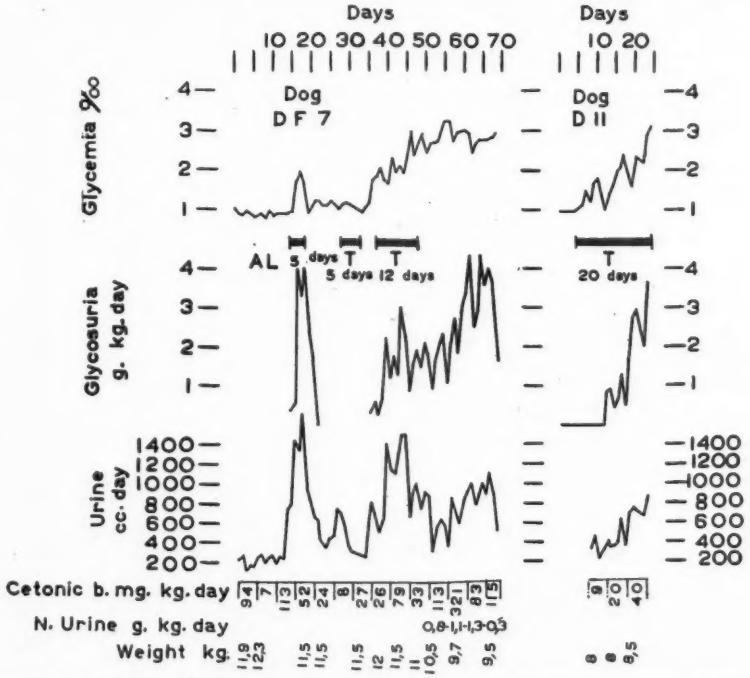

FIG. 2. Metathyroid diabetes (Dog DF 7) and thyroid diabetes (Dog D 11) produced by ingestion of 0.5 gm. per kg. per day of powdered bovine thyroid.

given thyroid for only 5 and 6 days; the other 5 had 3 gm. pancreas left and were given 0.5 gm. per kg. daily for 10, 16 and 20 days (perhaps the treatment was not sufficiently prolonged). Other animals died after a short time, these are not included. Yriart did not observe diabetes following thyroid feeding, due to the short time of treatment; 4 of his dogs received 0.4 to 0.6 gm. per kg. daily during only 6 to 8 days, and no significant variations in blood sugar were recorded; nevertheless in one animal a maximal blood sugar of 138 mg.% was seen after a first treatment and 168 mg.% on a second treatment; probably a permanent diabetes would have followed if thyroid feeding had been continued for a longer time.

Two subtotally pancreatectomized dogs showed great resistance

[3] To this series 2 thyroidectomized and 2 adrenal demedulated animals should be added.

TABLE 2. METATHYROID DIABETES PRODUCED BY INGESTION OF THYROID POWDER IN DOGS 8 TO 12 KG. WEIGHT, WITH NORMAL BLOOD SUGAR, SUBTOTALLY PANCREATECTOMIZED, LEAVING 2.5 TO 3 GM. PANCREAS. DOG DF 7 HAD 4 G. PANCREAS

Dog	Thyroid powder gm/kg/day	Number of days treatment	Days following operation before treatment	Hyperglycemia	Permanent diabetes	Max. diabetic glycemia mg. %	Death in days
H 1	0.5	15	15	yes	yes	323	27[4]
H 2	0.5	24	24	yes	yes	341	19[4]
DF 7	0.5	5	28	no	—		
	0.5	5[1]	103	yes	—		
	0.5	12	112	yes	yes	320	36
D 6	0.5	7[1]	201	yes	yes	380	63
D 8	0.5	8	68	yes	—	195	
	0.5	5[1]	82	yes	—	192	
	0.5	10	90	yes	yes	360	35
D 11	0.5	20	14	yes	yes	320	23
H 6a	0.5	21[1]	164	yes	yes	335	27
H 7	0.5	17[1]	103	yes	yes	340	17[4]
H 8	0.5	25	87	yes	yes	328	21[4]
H 9	0.5	15[1]	28	yes	yes	330	16
H 10[2]	0.5	19	34	yes	yes	360	21
H 11[3]	0.5	21	39	yes	yes	330	24
H 6	0.5	13	50	no	—	—	
	1.0	19	63	yes	yes	314	7[4]
H 5	0.5	13	33	no	—		
	2.0	9	46	yes	yes	286	16
H 4	0.5	32	345	no	—		
	1.0	39	377	no	—		
	2.0	9	385	yes	yes	345	16[4]
H 3	0.5	27	35	no	—		
	1.0	19	51	no	—		
	2.0	13	70	yes	no	114[5]	75[4]

[1] Shortly before had been treated with anterior hypophyseal lobe for 3 days and had a transitory diabetic reaction.
[2] Castrated male.
[3] Castrated female.
[4] Killed.
[5] Not diabetic, normal blood sugar.

to thyroid treatment, they had only moderate symptoms and no diabetes in spite of receiving doses of 1 and 2 gm. per kg. daily; one of these is Dog H3 in Table 2.

The prolonged period during which the animals had maintained a normal blood sugar before thyroid feeding began, excludes the possibility of a spontaneous diabetes appearing apart from any thyroid effect. At the time the experiments on thyroid feeding were being made 9 controls were kept in the laboratory for 5 to 9 months, following a subtotal pancreatectomy performed in the same way as in the thyroid fed dogs. None of these had diabetes, their blood sugar remained normal throughout the whole period.

The results obtained are specific for thyroid powder, since hyperglycemia and diabetes did not occur in dogs with a 2.5 to 3 gm. fragment of pancreas, when 2 gm. per kg. daily were given for one month, of the following powdered bovine tissues: muscle (2 dogs), kidney (2 dogs) and liver (2 dogs).

Rôle of other glands.—Diabetogenic action of the thyroid takes place in the absence of the gonads (Dogs H 10, male castrate and H 11 female castrate). The adrenal medulla is not a necessary factor in this action. In 4 animals the left adrenal medulla and the right adrenal gland were removed. Two of these had a metathyroid diabetes, the blood sugar rose after 20 and 26 days of treatment (0.5 gm. per kg. daily), to 320 and 348 mg.%; death occurred after 12 and 18 days of diabetes. The other 2 had no hyperglycemia after 32 days thyroid feeding; circumstances did not permit a more prolonged observation.

The thyroid is not a necessary factor in the diabetogenic action of thyroid feeding. A metathyroid diabetes was obtained in 2 previously thyroidectomized dogs recently (20 and 21 days) subtotally pancreatectomized. After 17 and 24 days of feeding thyroid powder (0.5 and 1 gm. per kg. daily) the blood sugar rose and they remained permanently diabetic.

Hypophysectomized dogs given 0.5 gm. per kg. thyroid daily, died 5, 7, 9, 10 and 13 days later. The blood sugar decreased in all and was around 70 mg.% after 15 hours' fasting, having been 80 to 95 mg.% before the thyroid treatment. The animals were greatly depressed, they had diarrhea and 3 died in convulsions due to hypoglycemia. Hypophysectomized rats also were sensitive to thyroid feeding, they died after a few days of receiving doses (10 to 25 mg. per 100 gm.) well tolerated by the controls; hypoglycemia and great loss of liver and muscle glycogen were observed.

Adrenalectomized rats were also found very sensitive to thyroid treatment, they died in hypoglycemia and showed decrease in muscle and liver glycogen. The right adrenal was removed from 4 dogs; 25 to 30 days later subtotal pancreatectomy was performed; after 20 to 25 days 0.5 gm. per kg. thyroid was fed daily and when the blood sugar had risen to 115–120 mg.% the left adrenal was extirpated. Thyroid feeding was continued, 10 mg. desoxycorticosterone acetate was injected subcutaneously every day together with 500 cc. 0.9% NaCl solution; 400 ml. milk with 5 gm. NaCl and 10 gm. sucrose were given by stomach tube. On the day following the second adrenalectomy the blood sugar rose to 124–139 mg.%, but afterwards decreased to subnormal values; 2 animals died in 3, and 2 in 4 days.

Action of insulin.—Thyroid was fed to 5 dogs; when the blood sugar had been above normal for 6 days (180 to 240 mg.%) thyroid was discontinued and 15 to 20 units insulin, divided in 2 doses, were injected subcutaneously every day. In all the animals the blood sugar returned to a normal level. After 15 days treatment, the insulin was discontinued, 3 dogs remained with a normal blood sugar level but the other 2 had a Sandmeyer diabetes which gradually became worse and killed the animals. Dogs H 9, H 10 and H 11 were left as controls and remained diabetic after discontinuing the thyroid feeding on the 6th day of hyperglycemia (blood sugar above 150 mg.%).

In another series dogs with a metathyroid diabetes of more than

two weeks standing and blood sugar above 250 mg.% were treated with insulin. The blood sugar returned to normal, but on discontinuing the insulin injections diabetes returned in its previous severity. In these cases no permanent cure was observed.

ASSOCIATION OF THYROID AND ANTEROHYPOPHYSIS

Six dogs subtotally pancreatectomized (3 to 4 gm. pancreas remaining) were alternatively treated with: a) thyroid feeding (0.5 gm. per kg. daily for 5 days); b) anterior lobe of bovine hypophysis (0.2 gm. per kg. daily intraperitoneally for 3 to 4 days); c) both treatments simultaneously. Thyroid treatment did not produce significant variations in blood sugar except in one dog in which it rose transitorily to 129 mg.%. The associated treatment produced greater and more prolonged hyperglycemia than the anterohypophyseal treatment given alone; 3 of the 6 animals remained with a permanent diabetes. Ketonuria increased from 3–8 mg. to 25–29 mg. per kg. per day with thyroid feeding; from 5–9 mg. to 6, 11, 20 and 69 mg. per kg. per day with anterohypophyseal treatment, and from 3–7 mg. per kg. per day to 14–42 mg. per kg. per day with the associated treatment; animals with severe diabetes eliminated 163 to 290 mg. per kg. daily. Diuresis was also greater when the animals received the associated treatment.

Dogs with intact pancreas received 1 gm. per kg. of bovine anterohypophysis daily for 5 days; in 13 out of 17 experiments the blood sugar was higher than when this treatment was associated with thyroid feeding (0.25 gm. per kg. daily).

Another series of dogs with intact pancreas received at the same time 0.5 gm. per kg. thyroid by stomach tube and 0.6 gm. per kg. fresh bovine anterohypophysis intraperitoneally. The association was evidently toxic and high mortality occurred: 1 animal died on the 2nd day, 3 on the 3rd, 1 on the 4th, 2 on the 6th, 1 on the 7th, 1 on the 8th, 2 on the 9th and 4 were killed on the 14th, 24th, 25th and 27th day. Only one of these last 4 had a permanent diabetes, another resisted treatment 27 days without hyperglycemia and symptoms.

A third series of 12 dogs with intact pancreas was injected for 4 days with anterior hypophysis (1 gm. per kg. daily) and on the following 10 days was fed 0.5 gm. per kg. thyroid daily; both treatments were repeated in the same order several times. The dose of anterohypophysis was at first 1 gm. per kg. daily and then reduced to 0.6 gm. per kg. when the blood sugar rose to 150 mg.% or more. Only 2 of these animals had a permanent diabetes after the 21 and 37 days treatment, 2 resisted the treatment and the rest died. The association of thyroid and anterohypophysis significantly reenforces the diabetogenic activity of each treatment.

Insulin secretion.—The intact pancreas of 3 dogs with a permanent metathyroid diabetes were grafted into blood vessels of the neck of diabetic dogs, pancreatectomized 24 hours before. No insulin secretion could be observed (Dog DF 12, figure 3). On the other hand the

pancreas from the resistant dogs appeared to secrete insulin in excess, since the blood sugar of the diabetic receptors returned to normal in 2 hours, instead of 3 hours, as occurred in the normal controls (Dog DF 20, figure 3).

These experiments show that the diabetogenic effects of the an-

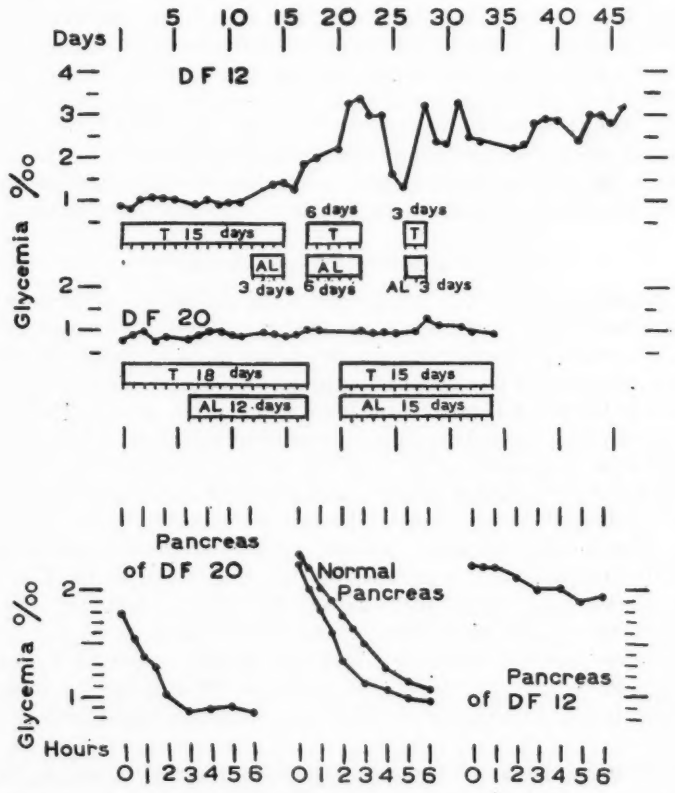

FIG. 3. Permanent diabetes produced by treatment with thyroid and anterohypophysis (Dog DF 2). Dog DF 20 did not respond to the same treatment.

Below: Vascular graft into the neck of diabetic dogs, pancreatectomized 24 hours previously, of the pancreas from DF 20 (resistant to treatment), 2 normal dogs and DF 12 (with permanent diabetes).

terohypophysis and the thyroid summate, but the toxicity of the association is very high.

EFFECT OF INGESTION OF THYROID ON DIABETES

The effect of thyroid feeding (0.25 to 1 gm. per kg. daily) was observed in 6 diabetic dogs: 2 had a Sandmeyer diabetes, 3 had a metahypophyseal diabetes and 1 had a metathyroid diabetes. All were in

good condition and were receiving 10 units insulin daily. Thyroid feeding (0.5 to 1 gm. per kg. daily) for 5 to 7 days produced an immediate and severe increase in the symptoms and killed the animals in 11 to 14 days. This effect commenced during the period of thyroid feeding and persisted after it had been discontinued; in some cases it was controlled by increasing the dose of insulin. The animals became depressed and weakened, they had diarrhea and a dirty aspect. In animals with a blood sugar of 200 mg.% a further increase was observed; in those with 300 mg. there was no increase in blood sugar. Glycosuria and a marked increase in diuresis were seen during the thyroid feeding and persisted during the 5 to 10 following days. Ketonuria increased in the 2 cases observed from 22 and 30 mg. per kg. daily to 188 and 155 mg. per kg. daily. There was a loss of 500 to 800 gm. body weight; in some cases even more. Insulin requirement was increased.

THYROID AND METATHYROID DIABETES

Thyroid diabetes.—The symptoms of the diabetes observed during thyroid feeding were those of all diabetes. Special features noted were: a) a very large diuresis; b) rapid loss in body weight; c) more insulin was needed to obtain a normal blood sugar. The increase in diuresis is the most typical sign; insulin resistance was well marked, but not as much as that produced by anterohypophyseal treatment.

Metathyroid diabetes.—The severity of the symptoms in metathyroid diabetes increased gradually until they were as severe as in pancreatic diabetes. Blood sugar in the last periods reached to 300–360 mg.%; glycosuria amounted to 2–5 gm., usually 3.5 to 4 gm. per kg. daily in well fed animals, and somewhat less when they fed badly. Diuresis increased with glycosuria and was usually between 500 and 800 cc., but amounts of 1500 and 2000 cc. were observed on some days. Ketone bodies gradually increased from the normal value of 4.5 to 10 mg. per kg. per day to 30, 70, 100 and even 200 mg. per kg. daily in the final stages. At autopsy the liver was found enlarged, yellow and fatty.

Three animals were killed while the symptoms were severe. Liver glycogen was much diminished (100, 90 and 96 mg.%); muscle glycogen was normal or only slightly below (340, 480, 420 mg.%); blood lactic acid was at a high value within the normal limits (20, 18, 22 mg.%).

Basal metabolism was determined[4] in 3 animals when their blood sugar was normal and later when they had a severe metathyroid diabetes. An increase in the B. M. was observed, similar to that seen in pancreatic diabetes (Table 3).

Rôle of the liver.—The liver was extirpated in 5 dogs with severe metathyroid diabetes. The blood sugar fell as in other experimental diabetes (figure 4).

[4] Dr. Mazzocco made these determinations.

TABLE 3. DOGS WITH SUBTOTAL PANCREATECTOMY, LEAVING 2.5 TO 3 GM. BASAL METABOLISM BEFORE AND AFTER PANCREAS PROVOKING METATHYROID DIABETES

Dog	B. M. when blood sugar normal. Average of 2 or 3 tests. Cal m²/h	Thyroid treatment		Duration of diabetes days	B. M.	Increase %
		gm/kg/day	during days			
H 8	860 cal. m² hour	0.5	25	16	994	14
H 1	910	0.5	15	22	1240	36
H 2	795	0.5	24	16	1020	30

Glucose tolerance tests.—In 5 dogs with a subtotal pancreatectomy (2.5 to 3 g. pancreas remaining), with normal blood sugar and when very quiet, 1 g. glucose per kg. was injected into the jugular vein and the blood sugar determined in blood taken from the ear. Thyroid (0.5

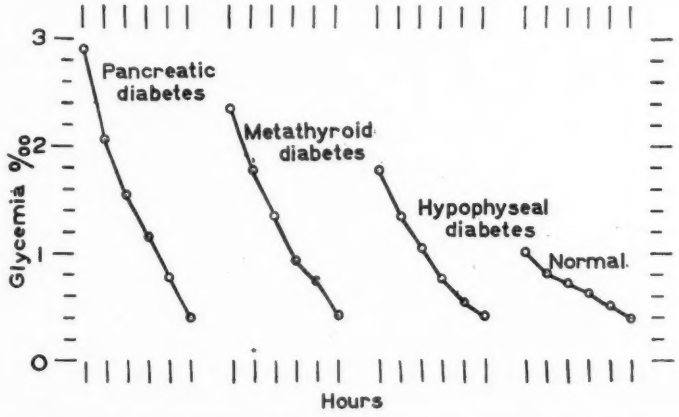

FIG. 4. Decrease in blood sugar following hepatectomy in chloralosed dogs. Average of 4 pancreatic diabetes, 5 metathyroid diabetes, 4 hypophyseal diabetes, 5 normal.

gm. per kg. daily) was fed for 5 days and the sugar tolerance curve again determined. The return to normal was slower after treatment, therefore the capacity of storing or utilizing glucose was diminished (figure 5).

Another series of 5 dogs had the sugar tolerance curve determined before thyroid feeding (normal blood sugar) and when in severe metathyroid diabetes 8 and 13 days after discontinuing the thyroid feeding. These last were typical diabetic curves (figure 6).

The 5 animals in the first series were injected 1 unit insulin per kg. intravenously and the blood sugar curve was followed before and on the 4th and 11th day of thyroid feeding. On the 4th day the decrease in blood sugar was slightly less and on the 11th day even less, than on the first occasion before thyroid feeding. The last curves

showed a rapid recovery after hypoglycemia (figure 7). Nevertheless this decrease in sensitiveness to insulin is not so marked as that produced by anterohypophyseal extract.

In a series of 5 dogs 1 unit insulin was injected intravenously before thyroid feeding and when metathyroid diabetes was permanently established. The decrease in blood sugar (absolute and per cent) was greater in the diabetic condition than before thyroid treatment (figure 8).

THYROIDECTOMY IN DIABETES

Including the dogs studied with Dr. Yriart and Dr. De Finis the effect of thyroidectomy on diabetes has been observed in 21 animals:

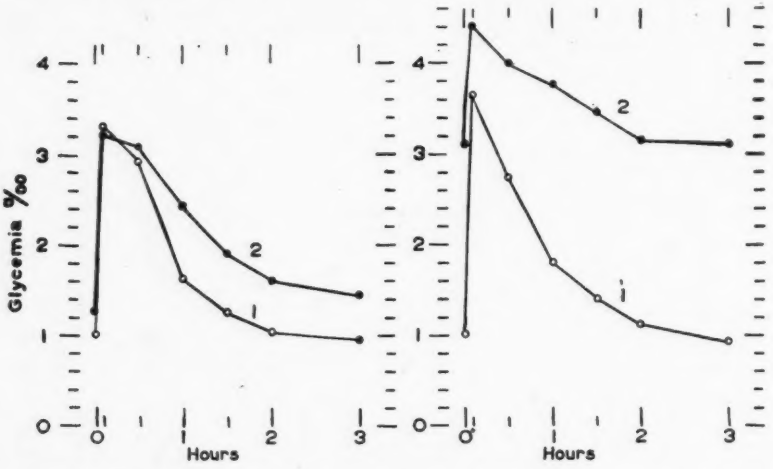

FIG. 5. Intravenous injection of 1 gm. glucose per kg.: 1, into 5 dogs with subtotal pancreatectomy and normal blood sugar. 2, into the same animals after 5 days of thyroid treatment (0.5 gm. per kg. daily).

FIG. 6. Intravenous injections of 1 gm. glucose per kg.: 1, into 5 dogs with subtotal pancreatectomy and normal blood sugar. 2, into the same animals in permanent metathyroid diabetes.

a) 5 had a Sandmeyer diabetes produced by subtotal pancreatectomy, b) 6 had a metathyroid diabetes; c) 3 had a metahypophyseal diabetes and 7 had a diabetes due to total pancreatectomy.

The animals in the first 3 groups were in hyperglycemia of 7 to 24 days standing when the thyroid was removed; 3 had blood sugar between 180 and 200 mg.%; the rest had blood sugar between 240 and 300 mg.%. In all these cases the operation exacerbated the symptoms: hyperglycemia, glycosuria and ketonuria increased. This postoperative reaction lasted 3 to 7 days; afterwards the diabetes progressed gradually and death occurred in 12 to 32 days (between 15 and 22 days in $\frac{2}{3}$ of the cases).

Thyroidectomized, totally pancreatectomized dogs were treated with insulin until the wounds had healed completely and they were in good general condition. At this time insulin was discontinued and a pancreatic diabetes of the usual severity was observed; death occurred in 24, 21, 12, 9, 9, 7 and 3 days in 7 dogs without infection studied by Yriart.

All these animals had blood sugar between 280 and 360 mg.% and in the final stages usually above 300 mg.%; in rare cases 400 mg. and 500 mg.% blood sugar were seen. Glycosuria amounted to approx-

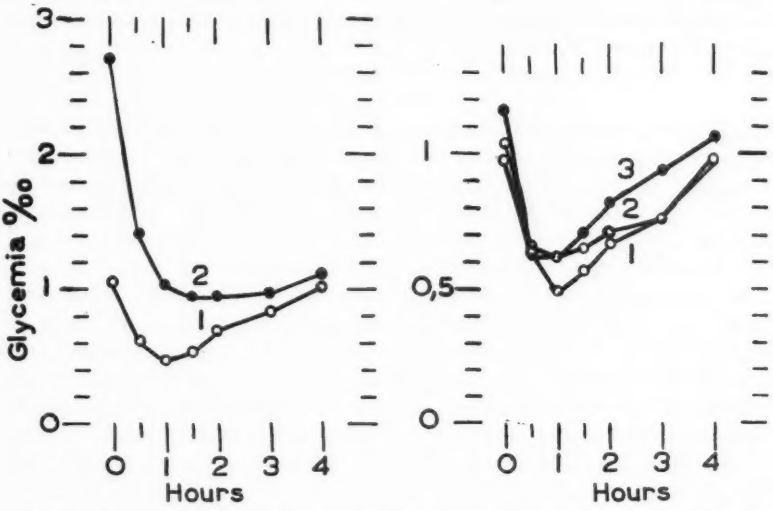

FIG. 7. Action of insulin; 1 unit per kg. intravenously. 1, in 5 dogs with subtotal pancreatectomy and normal blood sugar. 2, in the same animals in metathyroid diabetes.

FIG. 8. Action of 1 unit insulin per kg. intravenously. 1, in 5 dogs with subtotal pancreatectomy and normal blood sugar. 2, after 4 days thyroid treatment 0.5 per kg. daily. 3, after 11 days treatment.

imately 4 gm. per kg. daily, in concentration of 5.5 to 8.8 gm.%; decreasing when the animals eat badly but never disappearing completely. Nitrogen excretion increased by 1 to 1.7 gm. per kg. per day, usually by about 1.25 gm. per kg.; when feeding decreased, or stopped, it also decreased. The D:N ratio varied between 2 and 2.9. Ketone bodies increased up to 70, 100 and even 200 mg. per kg. daily. Autopsy showed the fatty liver usually found in pancreatic diabetes. Thyroidectomy did not reduce the severity of the symptoms in any of these experiments.

Three dogs with severe metathyroid diabetes received 10 units daily of insulin for 40, 30 and 65 days, with the object of decreasing glycosuria and maintaining a good general condition. When insulin

was discontinued diabetes reappeared in its previous severity and progressed till death occurred after 12, 16 and 16 days.

Thyroidectomy, according to personal experience, produces symptoms of thyroid insufficiency of less severity in the dog than in many other species, but nevertheless they are manifest. Basal metabolism decreases by 24% on an average; N excretion in fasting is 0.25 gm. per Kg. daily instead of 0.36 per kg. excreted by normal controls; in fasting animals after injection of phloridcin the N excretion rises to 0.63 gm. per kg. per day while in the controls it rises to 0.8 gm. per kg. daily. There is a marked retardation of growth in the thyroidectomized puppies. Phagocytic capacity of leucocytes is diminished by thyroidectomy (Parodi).

DISCUSSION

Thyroidectomy does not reduce the severity of diabetes in dogs. But this conclusion should not be extended to other species, since though in cat and dog attenuation of diabetes follows thyroidectomy, in man thyroidectomy diminishes glycosuria and the requirement of insulin. Observations on the rat will be published later.

These results are not surprising as in the dog and the cat diabetes runs a severe and rapid course; protein consumption is large and there is glucose formation from protein; hyperglycemia and glycosuria diminish but do not disappear during fasting. In the rat, diabetes progresses less rapidly, protein catabolism is not so intense and during fasting the blood sugar drops in a few hours to normal and even subnormal levels; at the same time glycosuria ceases. Hypophysectomy increases glucose consumption by the tissues in rats and rabbits, but not in dogs.

Thyroid feeding, even in large and toxic doses, does not produce diabetes in normal dogs. A few animals had slight damage in the islets, some of the β cells were abnormal. The pancreas from these animals in some cases could not respond with an increase in insulin secretion when submitted to the stress of lowering a diabetic hyperglycemia (experiments with pancreas grafted into the blood vessels of the neck). Thyroid feeding damages the islets when given in large doses to animals with a pancreas surgically reduced, and there are therefore a smaller number of islets, some of which are also damaged. Previously damaged islets are very sensitive to thyroid treatment; this is illustrated by the ease with which thyroid feeding produces diabetes in animals which have had a transitory diabetes provoked by anterohypophyseal or thyroid treatment. In order to sensitize the islets to the diabetogenic action of thyroid, the reduction of pancreatic mass must be considerable, no more than $\frac{1}{6}$ or $\frac{1}{8}$ of the total weight must remain (2.5 to 3 gm. in dogs weighing 7 to 10 kg.).

Removal of $\frac{4}{5}$ of the pancreas (4 g. remained) permitted the production of a permanent diabetes by thyroid treatment in 2 out of 6

experiments. Thyroid feeding did not produce diabetes in 2 dogs in which ½ the pancreas has been removed.

The pancreas of subtotally pancreatectomized controls not fed thyroid showed some normal islets, a few hypertrophied and some with more or less severe alterations in the β cells.

Diabetes produced by thyroid feeding can be of short duration; in these cases on discontinuing the treatment blood sugar quickly returns to a normal level. When thyroid feeding is prolonged diabetes becomes permanent and there is no recovery when the treatment is stopped. Thyroid diabetes is the name given to diabetes occurring in the course of thyroid feeding. Metathyroid diabetes is that observed after thyroid feeding has been discontinued. It is due to insufficiency of the islets produced by the treatment, which is no longer necessary to maintain the diabetic state.

The diabetogenic action of the thyroid is not so great as that of alloxan or anterohypophyseal extract. It is not diabetogenic in animals with an intact pancreas, but becomes so when the pancreatic mass has been diminished or the islets are already damaged.

The Langerhans islets show the usual alterations seen in diabetes. The β cells at first lose their granules and increase in size, then they suffer hydropic degeneration and finally become picnotic and die. When the treatment is stopped while only the two first stages have developed the β cells can recover; early treatment with insulin helps this recovery. When damage to the islets is severe and the animal remains in hyperglycemia the β cells gradually disappear, the islets become atrophied and connective tissue fibers are very prominent. In the few (5) cases examined the hyaline (or amyloid?) degeneration seen in some cases of metahypophyseal diabetes was not found.

The diabetogenic action of the thyroid was observed after removal of the gonads, the thyroid and the adrenal medulla. It could not be obtained in hypophysectomized or adrenalectomized dogs kept alive with hormonal treatment. These animals seem to be very sensitive to the toxic effects of thyroid feeding and the blood sugar and liver glycogen fell rapidly to low levels.

The diabetogenic activities of thyroid and anterohypophysis summate in dogs with a reduced or even an intact pancreas, whether they be given alternately or simulatenously. This association has a high toxicity.

The intact pancreas of dogs in metathyroid diabetes did not secrete insulin when they were grafted into the neck of diabetic pancreatectomizd dogs.

Thyroid feeding increases the severity of an existing diabetes and shortens the survival of the animals. There is considerable increase in diuresis; ketonuria and frequently glycosuria also increase; the blood sugar rises if it is not already at a high level; weight rapidly decreases and the insulin requirement is increased.

Metathyroid diabetes progresses as a Sandmeyer diabetes and in the last period shows the maximal severity in symptoms seen in diabetes produced by total pancreatectomy. Basal metabolism increases, blood sugar curves following glucose injection have the typical diabetic aspect, and the animals respond to insulin. The sugar in blood and urine come from the liver since hepatectomy is followed by a rapid fall in blood sugar to hypoglycemic levels.

SUMMARY AND CONCLUSIONS

1.—Prolonged administration of thyroid does not produce diabetes in dogs with an intact pancreas. It does produce it in dogs with only a fragment of 2.5 to 3 gm. pancreas left by a subtotal pancreatectomy, but with normal blood sugar. Previous treatment with anterohypophysis or thyroid sensitizes these animals to the diabetogenic action of thyroid.

2.—Diabetes so produced may be observed either only during the thyroid feeding (thyroid diabetes), or it may persist permanently after stopping the treatment (metathyroid diabetes). In both cases the β cells of the islets are damaged, reversibly in the first, permanently in the second. Insulin secretion stops as is shown by experiments in which the pancreas was grafted into the necks of pancreatectomized dogs. Insulin protects the islets during the first stages; later it is no longer efficient.

3.—Metathyroid diabetes can be obtained after removal of the gonads, thyroids and adrenal medulla. It could not be obtained in hypophysectomized and adrenalectomized dogs, because they died in hypoglycemia when given thyroid.

4.—Thyroid feeding increases the severity of slight or advanced diabetes; symptoms are exacerbated (diuresis, ketosis, loss of body weight, egt.) and the progress of the disease is accelerated, shortening survival.

5.—Metathyroid diabetes has the same characteristic as pancreatic diabetes: hyperglycemia, glycosuria, ketonuria, fatty liver, increase in protein catabolism, high basal metabolism, the same glucose tolerance curve, sensitiveness to insulin. Hepatectomy produces a fall in blood sugar to hypoglycemic level.

6.—In dogs, thyroidectomy does not modify diabetes produced by total pancreatectomy, nor Sandmeyer's, metahypophyseal and metathyroid diabetes.

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INFLUENCE OF REPRODUCTIVE HORMONES UPON GROWTH IN OVARIECTOMIZED AND NORMAL FEMALE RATS

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CONTRIBUTIONS to the knowledge of the influence of reproduction, especially that of the individual reproductive hormones upon the growth rate in the female, have not been numerous. In most of these investigations the effects of ovariectomy or of pregnancy were generally studied.

Evidence that ovariectomized rats grow more rapidly than virgin controls has been presented by Stotsenburg (1913), Slonaker (1930), Freudenberger and Billeter (1935), Freudenberger and Howard (1937), Freudenberger and Hashimoto (1937–38 and 1939) Bogart *et al.* (1940), and Tang (1941). Pronounced differences in the rate of growth were shown to exist between pregnant and virgin female rats of the same age by Slonaker (1927), Cole and Hart (1938) and Bogart *et al.* (1940). The effects of pregnancy, pseudopregnancy (Hart and Cole, 1939) and ovariectomy upon growth indicate that the reproductive hormones may be directly or indirectly involved.

Investigations have been made of the effect of injected estrogens on growth; but in most cases large quantities of the hormone were given, and the primary concern was the influence of the hormone upon skeletal changes—Bugbee and Simond (1926), Spencer, Gŭstavson and D'Amour (1931), Zondek (1936), Freudenberger and Clausen (1937), Gardner and Pfeiffer (1943). Fewer studies of the effect of injected progesterone on growth have been made, and in the two reports found in the literature, Gallardo (1940) and Selye (1940), large quantities of the hormone (2 to 5 mg. daily) were injected.

In an endeavor to ascertain the influence of the individual reproductive hormones upon the growth rate of ovariectomized and normal female rats, the present investigation was made, in which small quantities of injected estrone and progesterone were employed and the effects of increased progesterone production induced by pseudopregnancy or luteinizing hormone were studied.

MATERIALS AND METHODS

All rats used in this investigation were fed a grain ration supplemented with whole milk, minerals, and vitamins A and D. The animals were ran-

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domly distributed throughout the cages with a maximum of 6 rats to a cage. Both progesterone[2] and estrone[2] were dissolved in peanut oil and subcutaneous injections were made daily. The concentration of progesterone was 0.5 mg. per cc. and that of the estrone was 100 I.U. per cc. In those animals receiving either hormone alone, 0.1 cc. of the oil solution was injected daily, while in those receiving both hormones, 0.1 cc. of each solution was administered. The quantities of chorionic gonadotropin[3] given (8 R.U. every 5 days) were based upon assays of the company which supplied the material. All rats were individually weighed to the nearest gram at 10-day intervals. Determinations of moisture and fat were made on the carcasses of progesterone-injected, estrone-injected, and ovariectomized rats and comparisons were made with the controls. The moisture was determined on 10 gm. samples of ground carcass by drying to constant weight in a vacuum at room temperature. In preparing the tissues for moisture determination the skin and intestinal tract were removed, but all other tissues were ground and thoroughly mixed. The fat determinations were made on samples of the dried ground carcass by extractions with hot alcohol and ethyl ether.

RESULTS

The influence of injected progesterone and estrone upon growth rate of ovariectomized and unoperated female rats. Two groups of female

TABLE 1. INFLUENCE OF PROGESTERONE AND ESTRONE UPON BODY WEIGHT OF OVARIECTOMIZED AND NORMAL FEMALE RATS

Treatment	Number of animals	Body weight in grams			
		Initial	1 Month	2 Months	3 Months
Ovariectomized	16	37	114	168	193
Normal	16	37	116	164	185
Progesterone[1]	16	37	117	169	192
No-progesterone	16	37	113	163	186
Estrone[2]	16	37	111	156	179
No-estrone	16	37	119	177	199
Necessary Difference[3]		1.1	9.3	7.1	7.3
Ovariectomized	4	37	122	185	207
Ovariectomized + Progesterone	4	37	117	183	206
Ovariectomized + Progesterone + Estrone	4	38	112	155	186
Ovariectomized + Estrone	4	38	104	150	174
Normal	4	39	114	159	175
Normal + Progesterone	4	37	122	179	207
Normal + Progesterone + estrone	4	37	117	164	183
Normal + Estrone	4	37	110	153	175
Necessary Difference[3]		2.6	16.7	19.7	21.3

[1] Progesterone in peanut oil. 1/20 mg. per rat per day.

[2] Estrone 1 R.U. per rat per day.

[3] Calculation and Interpretation of Analysis of Variance and Covariance. G. W. Snedecor, Collegiate Press, Inc., 1934.

[2] The estrone and progesterone were supplied by Parke, Davis and Company through the courtesy of Dr. Oliver Kamm.

[3] The chorionic gonadotropin, Korotrin, was supplied by Winthrop Chemical Company Inc. through the cooperation of Dr. Charles E. Fanslau.

rats were used. The first group of 32 animals averaging 37 gm. was comprised of 16 ovariectomized[4] and 16 unoperated control rats. Eight of the ovariectomized rats and 8 of the controls were administered progesterone; while the remainder of the operated and control rats received no progesterone. Four of the progesterone-treated females and four of those receiving no progesterone received daily injections of the estrone in oil. By this division of the group it was possible to compare the 16 receiving progesterone with the 16 re-

TABLE 2. INFLUENCE OF PROGESTERONE AND ESTRONE UPON BODY WEIGHT OF OVARIECTOMIZED AND NORMAL FEMALE RATS

Treatment	Number of animals	Body weight in grams			
		Initial	1 Month	2 Months	3 Months
Ovariectomized	12	117	187	200	219
Normal	12	118	166	178	190
Progesterone[1]	12	117	181	194	211
No-progesterone	12	117	172	184	198
Estrone[2]	12	118	173	185	196
No-estrone	12	118	180	193	215
Necessary Difference[3]		2.7	9.2	9.1	9.7
Ovariectomized	3	116	186	197	220
Ovariectomized + Progesterone	3	116	202	220	245
Ovariectomized + Progesterone + Estrone	3	117	179	188	203
Ovariectomized + Estrone	3	117	182	194	209
Normal	3	117	164	174	183
Normal + Progesterone	3	119	169	181	203
Normal + Progesterone + Estrone	3	118	172	185	193
Normal + Estrone	3	116	159	173	181
Necessary Difference[3]		4.1	10.7	12.9	13.3

[1,2,3] Footnotes as in table 1.

ceiving none, the 16 ovariectomized rats with the 16 unoperated rats, and the 16 receiving estrone with those receiving none.

The second group of 24 female rats averaging 117 gm. in weight consisted of 12 ovariectomized[5] and 12 unoperated rats which were subdivided into smaller groups and treated with the hormone preparations in the same manner as the 32 rats of the first group. In this group it was possible to compare the 12 receiving progesterone with the 12 receiving none, the 12 ovariectomized with the 12 unoperated rats, and the 12 receiving estrone with the 12 receiving no estrone.

The first group of rats with the lower initial weight of 37 gm. shall be designated as the group of smaller rats and the data on their body weights are presented in table 1. The group of rats averaging 117 gm. initial weight shall be called the group of larger rats and the data on them are presented in table 2.

[4] Ovaries removed when rats averaged 37 gm. in weight.

[5] Ovaries removed when the average weight of the rats was 117 gm.

It is shown (tables 1 and 2) that the ovariectomized animals of both groups were significantly heavier than the unoperated controls. In the group of smaller rats this difference in weight was not significant until the rats had been on treatment for 3 months, whereas in the group of large rats the difference was significant ($P = 0.05$) one month after the operation.

The average weight of the rats receiving progesterone was greater than that of those receiving none. In the group of larger rats (table 2) this difference was significant after the rats were treated for 2 months, while in the group of smaller rats (table 1), although a difference in weight existed, it was never highly significant.

The animals receiving estrone grew less rapidly than those which did not receive this hormone. The weight difference between those receiving estrone and those receiving none was highly significant in the group of smaller rats (table 1) after treatment for 2 months and in the group of large rats (table 2) after treatment for 3 months. In both groups (tables 1 and 2) it is evident that the estrone produced a greater retardation in growth in the ovariectomized rats than in their unoperated controls. On the other hand, the growth response of the ovariectomized and normal rats to progesterone treatment was approximately the same. In the group of large animals progesterone accelerated growth rate to a greater extent than in the group of smaller rats, and this was particularly striking in the ovariectomized animals. Normal animals receiving both progesterone and estrone were heavier than those receiving no treatment or those receiving the estrone alone, but weighed less than the normal rats receiving the progesterone alone (tables 1 and 2). Ovariectomized rats receiving both progesterone and estrone showed a growth response similar to that of ovariectomized rats receiving estrone alone. The operated animals injected with both hormones were also smaller than those on progesterone alone or those receiving no treatment.

Since the above data have shown that progesterone, ovariectomy and estrone have an influence upon the growth rate, it was deemed of importance to determine whether these differences in growth rate were the result of normal growth changes or the result of increased deposition of fat and/or water. The relative proportions of fat and water of 8 representative animals from each group of experimental animals, as designated in table 6, were determined. The data (table 6) show no pronounced differences in the percentage of fat and water which might be associated with the type of treatment. The group variation in percentage of water ranged from 68.4 to 70.1 per cent and the variation in fat ranged from 14.8 to 16.1 per cent. These small group differences in the proportion of fat and water indicate that no excessive deposition of either occurred.

The quantity of estrogen required to retard the growth rate of ovariectomized rats to that of normal controls. Different levels of estrone were

TABLE 3. THE QUANTITY OF ESTRONE REQUIRED TO RETARD THE GROWTH RATE OF OVARIECTOMIZED RATS EQUAL TO THAT OF NORMAL CONTROLS

Treatment	Initial weight in grams	Body weight in grams following treatment			
		1 Month	2 Months	3 Months	Final 110 days
Ovariectomized control 6 rats	121	189	205	224	225
Ovariectomized 3 rats—10 I.U.—every 8 days 3 rats—10 I.U.—every 4 days	122	177	196	202	207
Ovariectomized 3 rats—20 I.U.—every 4 days 3 rats—40 I.U.—every 4 days	121	176	187	192	195
Ovariectomized 3 rats—80 I.U.—every 4 days 3 rats—160 I.U.—every 4 days	122	171	186	188	190
Controls Normal virgin 6 rats	121	165	178	191	190
Necessary Difference	3.8	11.3	11.6	11.1	11.7

injected into ovariectomized rats to determine the quantity of hormone necessary to decrease their growth rate to that of the normal controls. The weights, at the beginning and after treatment for different lengths of time, of animals receiving different amounts of estrone are compared with ovariectomized and normal animals receiving no estrone in table 3.

It is apparent (table 3) that the ovariectomized animals receiving no estrone gained much faster than those injected with the hormone. They also grew at a more rapid rate than their unoperated controls. Injection of estrone at a rate of 20 to 40 I.U. per rat every 4 days decreased the rate of growth of these animals to the extent that their final weight approached that of the controls. The injection of 80 to

TABLE 4. INFLUENCE OF PSEUDOPREGNANCY UPON GROWTH RATE IN RATS

Age	Groups*	
	A	B
	Virgin controls	Vasectomized ♂ with ♀ ♀ when they were 80 days old
24 days	33 gm.	34 gm.
52 days	108 gm.	104 gm.
80 days	152 gm.	153 gm.
108 days	182 gm.	201 gm.
136 days	198 gm.	217 gm.
164 days	213 gm.	228 gm.

* 4 ♀ rats in each group.

160 I.U. per rat every 4 days held the weight of the operated females near that of the normal controls (table 3).

Influence of increased progesterone production on growth. Four female rats were used as controls while 4 others were mated to a vasectomized male. This sterile male was with the 4 females from the time they averaged 80 gm. until the experiment was terminated. The final weights of mated females were approximately ten per cent heavier than those of the controls (table 4).

TABLE 5. GAIN IN WEIGHT OF VIRGIN FEMALE RATS AS INFLUENCED BY PREGNANCY, LUTEINIZING HORMONE, AND PROGESTERONE (60 DAYS)

Number of animals	Treatment	Initial weight	Final weight	Total gain	Gain per day
6	Bred, young destroyed at birth	194	255*	61	1.01
6	Virgins, luteinizing hormone, 8 R.U. every 5 days	196	217	21	0.36
6	Virgins, progesterone 0.143 mg. every 5 days	193	211	18	0.30
6	Virgin controls	195	199	4	0.07
	Necessary difference				0.29

* Non-pregnant weight, each female had 2 litters and matings were then discontinued.

When the growth of the virgin female rats used in this experiment had reached a stage corresponding to that represented by the plateau in the growth curve, 24 rats were selected and divided into 4 groups of 6 rats. The first group of 6 females was mated and bore 2 litters each, but they were not permitted to suckle the young. Throughout a period of 60 days the 6 rats of the second group were injected every 5 days with 8 R.U. of chorionic gonadotropin per rat, whereas those comprising the third group were each administered 0.143 mg. of progesterone in 0.1 cc. of peanut oil every 5 days by subcutaneous injection. The remaining 6 rats were kept as controls.

The gain per day is given in table 5. The animals that bore young but did not suckle gained weight more rapidly than non-mated fe-

TABLE 6. THE EFFECT OF ESTRONE, PROGESTERONE AND OVARIECTOMY UPON THE PERCENTAGE OF FAT AND WATER IN THE CARCASSES OF FEMALE RATS

Treatment	Number of animals	Average % fat in carcass	Average % of H^2O in carcass
Ovariectomized	8	15.3	68.1
Normal	8	15.7	70.1
Progesterone	8	16.1	68.4
No progesterone	8	14.8	68.7
Estrone	8	15.6	68.6
Noestrone	8	15.8	68.5

males which received the luteinizing hormone or progesterone and much more rapidly than the virgin controls (table 5). Although the females receiving the chorionic gonadotropin and progesterone gained more rapidly than virgin controls, the difference is not highly significant. There was no difference in rate of gain of female rats receiving luteinizing hormone and progesterone.

DISCUSSION

The results of this investigation (tables 1 and 2) confirm previous observations that ovariectomy results in a more rapid rate of growth in rats. However, the age at which the animals are ovariectomized influences the degree of response. Ovariectomy before puberty did not result in a more rapid rate of growth in the operated animals than that in the controls until both control and operated animals had reached the age of puberty when the ovaries begin actively to produce estrogen. On the other hand, if the animals were ovariectomized at or shortly following puberty, the differences in growth rate between the operated and control animals were immediately exhibited. Apparently, then, the greater growth resulting from ovariectomy can be attributed to the elimination of the inhibitory effect of estrogens. A similar concept was advanced by Silberberg and Silberberg (1941), who stated that ovariectomy eliminates estrogen effects on skeletal changes.

In general, injected estrogens retarded the growth rate as shown in tables 1, 2 and 3, but the retardation of the growth rate was more pronounced in the ovariectomized rats. Since it is generally accepted that estrogens inhibit the production of F.S.H., which is usually accompanied by a corresponding increase in the quantity of L.H., it might be that the difference in the response of normal and ovariectomized rats to injected estrone may depend upon the increase in progesterone through L.H. stimulation of the ovary in the normal animals.

The results obtained by the injection of estrone into ovariectomized and normal rats (tables 1 and 2) indicate that growth was retarded to a greater degree in the group of smaller rats. If the effect of estrogens on growth is a retardation of the rate, then retardation effects should be more pronounced at the time when the growth rate is at a maximum.

It has previously been suggested in this paper that the large quantities of estrogens used in previous growth studies may not be quantitatively comparable with that produced under normal physiological conditions. The authors assumed that the quantity of estrogen necessary to retard the growth rate of ovariectomized rats to that of the virgin controls should approximate the amount normally produced systemically by rats of similar weight and age. The data reported in table 3 indicate this quantity to be between 10 and 40 I.U. per rat per

day. On this basis, it appears that the injection of 10 I.U. per rat per day is within the physiological level for the rat.

In contrast to the effects of estrogen, progesterone accelerated the rate of growth. The effect of progesterone on growth rate was more pronounced in the larger rats, both normal and ovariectomized. As progesterone affects growth by an acceleration of growth rate, it may be assumed that the acceleration would be relatively greater at that stage in growth when the rate is at a minimum.

In pregnancy, pseudopregnancy and through the injections of the chorionic gonadotropin an increased production of progesterone occurs which is accompanied by an increased rate of growth. However, the growth rate during pregnancy in the rat was found to be greater than that induced by injection of progesterone or by the injection of luteinizing hormone, or by pseudopregnancy as shown by data reported herein and by Hart and Cole (1939) and Bogart *et al.* (1940). It is recognized that in pregnancy other factors exist which might influence the growth rate. One of these factors may be the increase in appetite accompanying pregnancy. Hart and Cole (1939) have shown, however, that appetite is not stimulated by injected progesterone, which might indicate that the increased appetite during pregnancy may be the result of factors other than the increased amounts of progesterone existing at that time.

In summary, whether injected or systemically produced, progesterone accelerates and estrone retards the rate of growth. The acceleration or retardation of the growth rate produced by small quantities of these hormones is not accomplished by altering the normal content of fat or water in the organism as demonstrated in the data obtained by analyses of the carcasses of the experimental animals.

SUMMARY

Estrone injected into pre-pubertal or post-pubertal female rats retards the growth rate of the normal and the ovariectomized animals of both groups. The degree of response to injected estrone is greatest in the pre-pubertal rats in which growth rate is at a maximum. The retardation effect of estrone is relatively greater in the ovariectomized rats than in the controls. Again the retardation effects of the estrone were greatest in that group possessing the more rapid rate of growth.

Data have been presented which show that the quantity of estrone necessary to retard the growth rate of ovariectomized post-pubertal rats to that of the unoperated controls is between 10 and 40 I.U per rat per day. The assumption was made, based on these results, that the quantities of estrone administered in the present investigation were within the physiological level of estrogen production of the post-pubertal virgin female rat.

In contrast to the estrone effects, progesterone accelerated the rate of growth. The relative rate of acceleration was apparently influenced

by the growth rate, as the greatest increases in growth occurred in those rats in which rate of growth was at a minimum. This effect of progesterone upon growth rate was observed whether the hormone was injected or was systemically produced in pseudopregnancy or pregnancy or by the injection of a luteinizing hormone. It was noted that pregnancy gave a more pronounced stimulus to growth than either injected or systemically-produced progesterone alone.

The insignificant differences in the fat and water content of the carcasses of rats from various groups in this investigation indicate that factors other than abnormal accumulations of either of these substances are responsible for the changes in growth rate produced by the hormones.

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TESTICULAR RESPONSE TO ANDROGEN IN THE LIGHT-STIMULATED STARLING[1,2]

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THE EFFECTS of androgens on the male reproductive system of a variety of vertebrates have been summarized by Moore and Morgan (1942). Most of the studies on warm-blooded animals have been made on those which reproduce continuously throughout the year. Recently, Wells (1943) has investigated the ground squirrel, and Pfeiffer (1944) the house sparrow. These animals have a more restricted breeding season than do most laboratory animals.

For a number of years the sexual cycle of the adult male starling has been intensively studied, principally in regards to the relation of the external environment to this cycle (cf. Bissonnette, 1937; Burger, 1940, 1943; Burger, Bissonnette, and Doolittle, 1942, for summaries). During the fall and winter sperm can be precociously induced by duplicating with artificial light the lengthening of the days which occurs during the winter and spring. Since 'long days' have been shown to be a powerful stimulus to the testis-activating mechanism, starlings which are subjected to stimulating light-treatments seem to offer good material for the study of the possible stimulating or depressing effects of androgens on the pituitary-testis system.

The present study deals with the effects of exogenous testosterone propionate on otherwise normal adult male starlings which were precociously stimulated sexually by artificial light. The results shed some light on two problems. 1) Whether or not the cessation of sperm production which occurs after the breeding season is due to an inhibition of the pituitary by the male sex hormone. 2) What role does male hormone play in the testis during the period when the testis-stimulating mechanism is activated.

MATERIALS AND METHODS

Only newly captured adult male starlings (*Sturnus vulgaris*) were used. Birds were selected for approximately the same weight and for the same degree of yellow coloration of the bill. It has been shown (Witschi and Miller, 1938) that this color is due to male hormone. It may be assumed that star-

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lings having bills which show the same amount of change from black to yellow are in a similar state of endogenous hormone production. Birds were kept in cages of uniform dimensions. They received light solely from artificial (Mazda) sources. Diet, temperature, and other environmental factors were uniform.

Two experiments (I, II) were conducted. The first ran from February 17 to March 24 (36 days). Three groups of 12 birds each were used. One group was placed on a daily light ration of 14 hours, and injected subcutaneously every 72 hours with 1.0 mg. of testosterone propionate in 0.1 cc. of sesame oil, for a total of 11 mg. for each bird. The control group received the same daily light ration with injections of oil only on the above schedule. The third group was placed on a daily light ration of 9 hours for the first 19 days, during which time 5 injections of 1.0 mg. each of testosterone propionate were made. After 19 days, the hormone treatment was stopped, and the daily light ration raised to 14 hours.

The second experiment ran from March 27 to April 24 (26 days). One group of 8 males received 8 mgs. of testosterone propionate in 1.0 mg. doses every 72 hours. Eight controls received oil alone. Both lots experienced 14 hours of daily illumination.

In the first experiment, birds were sacrified on March 7, 14, and 24; in the second experiment, on April 10 and 24. The testes were treated for histological study.

RESULTS

Experiment I. The macroscopic results of this experiment are shown in figure 1. At the start of the experiment (February 17) the

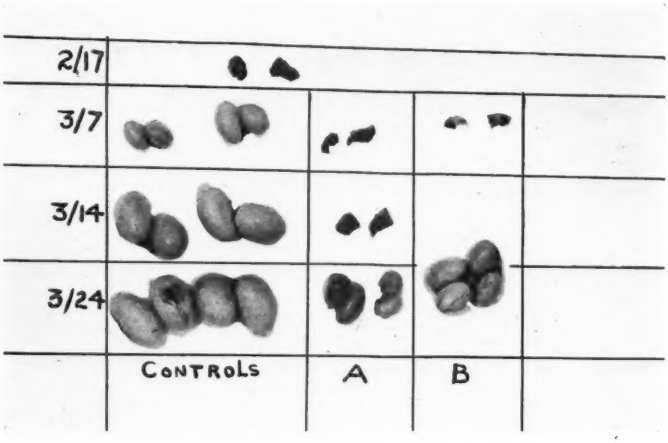

FIG. 1. Photographs of testes from experiment I. The testes A were from starlings treated with testosterone propionate and 14 hours daily light. Those at B and opposite 3/7 were from birds treated with androgen and 9 hours daily light. Those at B-3/24 were from the originally short-day birds after androgen withdrawal and the raising of the daily light ration at 14 hours. No birds were sacrificed at 3/14 in this latter group (B).

testes showed a slight activation. Only spermatogonia were present, but a multiplication of these cells had increased the size of the testis and the diameter of the tubules beyond the summer and fall condition of sexual quiescence.

The controls under the stimulus of light rapidly formed sperm. By March 17 abundant sperm was present. The testes of the birds which in addition to light received testosterone proprionate did not respond as did the controls. After 19 days of hormone treatment (March 7) the size of the testes and the diameter of the tubules had decreased. Histologically, the testes appeared inactive. After 26 days of treatment, the size of the testes and the diameter of the tubules had increased to approximately the same condition present at the start. After 36 days of hormone treatment a few spermatids were present. Approximately the same stage of testicular maturation was reached as that attained by the controls some 17 days earlier.

In the starling inter-tubular cells are few. In the sperm-producing testis there does not appear to be inter-tubular cells comparable to the interstitial cells of mammals. Not all birds however, show this dearth of inter-tubular cells. Hormone treatment produced no deviation from the controls in regard to the inter-tubular cells.

In the birds subjected to 9 hours of daily light and injected with testosterone propionate, the depressing effect of the hormone were more marked than for the birds which received longer illumination and hormone treatment. After 19 days of treatment the testes was minute. After these 19 days the hormone was discontinued and the daily light ration raised to 14 hours. The depressing effect of the hormone ceased with its withdrawal. For the remainder of the experiment, the testes matured at the same rate as did the controls but of course, they were 19 days tardy. The initial depressing effect observed was not due to the reduction of the daily light ration. Testes do not involute to so small a size when the light ration is reduced.

Experiment II. At the start of this experiment (March 27) natural sexual activation was well along. In the more advanced birds, sperms were already present. All were producing spermatocytes at the least. Controls placed on 14 hours daily of artificial light continued to produce sperm. Starlings which received testosterone propionate in addition to light continued their maturation at the same rate as the controls. In this experiment no depressing effects of testosterone propionate were observed.

DISCUSSION

In birds such as the starling where the inactive testis can be stimulated to produce sperm by the application of increased day-lengths, it is generally agreed on experimental and theoretical grounds that light stimulates the pituitary to produce or release the appropriate gonadotropins. The mechanism whereby this stimulation of the pi-

tuitary occurs is debated, but there is no evidence to indicate that light affects the testis directly. In the starling the amount of stimulation of the testis is quantitatively related to the light stimulus (Burger, 1940, 1943; Burger, Bissonnette, and Doolittle, 1942). In the mammal the relationship to light appears to be more loose, and more depends on an inherent cycle. In short, in the starling, 'long days' are a powerful stimulus to activation of pituitary-testis system, and short days are inhibitors to this system.

Under the conditions of the above experiments, different effects were secured with testosterone propionate. These differences were associated with the degree of activity of the gonad-stimulating system. When under light stimulation, the testes were well advanced into spermatogenesis, no effects of exogenous testosterone propionate were observed (experiment II). When hormone treatment was started before the testes had become very active, the hormone tended to nullify the stimulating effects of light, and apparently, even depressed the gonad-stimulating system so that the testes decreased in size and activity. Without the light stimulus, the testes became even smaller under hormone treatment. The androgen could not keep the gonad-stimulating system suppressed. Gradually testicular maturation occurred, but less rapidly than without hormone treatment or when the hormone was withdrawn.

In general the *initial* effect of the hormone on the starling which is relatively inactive sexually is similar to that recently described for the sparrow (Pfeiffer, 1944). In the sparrow, testosterone propionate produced within the first two weeks an increase in the size of the testis, followed by a return to the original size or smaller. Since our birds were not killed before the first 19 days, this effect, if it occurred, was not observed. The lack of effect on the sperm-producing testis is like that reported for the opossum (Moore and Morgan), and for the ground squirrel (Wells, 1943).

Although the hormone was administered to the starlings only every 72 hours, the doses (1.0 mg.) were fairly massive. The failure to inhibit spermatogenesis once it was actively occurring makes it seem unlikely that in the starling the gradual decline of spermatogenesis and the return to sexual quiescence which occurs after the breeding season, is due to the male sex hormone. This is assuming that male sex hormone of the starling is a material qualitatively similar to testosterone propionate.

SUMMARY

Testosterone propionate produced no stimulating effects on spermatogenesis in the adult male starling. Testes naturally in the later stages of spermatogenesis were unaffected by the androgen. Testes naturally in early spermatogenesis showed an initial depression in size and activity. This was more marked in birds on 'short days' than

in starlings treated with daily light rations which are otherwise stimulating to spermatogenesis. Under continued treatment with light and androgen, spermatogenesis occurred, but less slowly than without androgen. The cessation of spermatogenesis which normally occurs at the end of the breeding season does not appear to be produced by an androgen qualitatively like testosterone propionate.

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EFFECT OF THIAMIN DEFICIENCY AND CONTROLLED INANITION ON OVARIAN FUNCTION

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EXPERIMENTAL studies have shown that a diet deficient in the vitamin B complex or thiamin (vitamin B_1) will interfere with ovarian function and produce anestrous (Evans and Bishop, 1923; Parkes, 1928; Shin, 1933; and others). However it is not clear from these studies if the resulting anestrous was due to a deficiency of vitamin B complex, or due to a thiamin deficiency *per se*, or an effect of the concomitant inanition. In more recent studies Coward and Morgan (1941) observed that rats fed a thiamin-free diet had two or three normal cycles and then entered into anestrous. Again the effect may have been due to the thiamin deficiency *per se* or the resultant inanition.

Others have reported that partial or acute starvation will result in ovarian atrophy or loss of estrus in rats, which they attributed to insufficient pituitary gonadotropin secretion (Mulinos *et al.*, 1939; Mulinos and Pomerantz, 1940; Werner, 1939). No data is given, however, on the vitamin content of the diet or of the vitamin supplements.

In view of the above reports, and the often repeated statement that a deficiency of thiamin effects ovarian function, experiments were undertaken to determine whether the ovarian hypofunction was caused by the avitaminosis *per se* or by the inanition resulting from lack of the vitamin. The results offer evidence that a thiamin deficiency disrupts normal ovarian function in the rat because of the inanition produced by the loss of appetite.

PROCEDURE

Adult female white rats, of approximately uniform weight and having normal cycles were selected for the experiments. For a period of ten days the rats were fed a synthetic diet.[2] A specially prepared vitamin solution,[3] pro-

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[2] *Composition of Diet.*

Lard	15.0 gm.
Casein (vitamin-free)	25.0
Cornstarch	56.0
Salts (Osborne-Mendel)	4.0
	100.0 gm.

viding the factors of the B complex, was administered orally by tuberculin syringe in daily amounts of 0.2 cc. Oleum percomorphum was diluted with corn oil so that 0.1 cc. supplied 500 I.U. of vitamin A and 70 U.S.P. units of vitamin D. This amount was administered orally twice weekly by tuberculin syringe.

At the end of the ten-day period the rats were divided into two groups so that the average body weights of the two groups were equivalent. One group (control) was continued on the control regimen while the other group (thiamin-deficient) was given the same diet as the control group but with thiamin lacking in the vitamin supplement. The thiamin-deficient group received food *ad libitum*, but the food consumption for each rat in the control group was limited to the average quantity consumed by the thiamin-deficient group on the previous day.

RESULTS

Growth and estrous cycles.

Experiment I. In this experiment there were seven rats in each group. The food intake of the group which received no thiamin decreased over a period of thirty days (fig. 1). As the food intake of the thiamin-deficient animals decreased, the food intake of the control animals was so restricted that the daily caloric intake was the same in both groups. The body weight curves for the two groups were similar, first showing an increase in weight and then a marked decrease as the degree of anorexia increased.

On the thirty-first day of the experiment the thiamin-deficient animals were given the complete vitamin supplement instead of the thiamin-free solution. Food intake for this group immediately increased markedly, from a daily average of 0.8 gm. to 7.5 gm. On the following day and thereafter this group received the thiamin-free supplement as before, and the food intake gradually decreased again, reaching the previous low level nine days after the single administration of thiamin (20 gamma). The body weight, after this single administration of thiamin, increased and then decreased again in accordance with the changes in food consumption. Since the control group received the quantities of food dictated by the appetites of the thiamin-deficient animals, the fluctuations in body weight were similar in both groups.

Among the thiamin-deficient animals, estrous cycles ceased after sixteen to twenty-two days without thiamin (Fig. 1). All but one rat

[3] *Vitamin solution.* (Vitamin content per 0.2 cc. of daily supplement.)

Thiamin	20 gamma
Riboflavin	40 gamma
B_6	20 gamma
Ca pantothenate	100 gamma
Nicotinic acid	500 gamma
Biotin (S.M.A. 1000×conc.)	0.2 gamma
Inositol	1.0 mg.
Choline	5.0 mg.
p-amino-benzoic acid	1.0 mg.

showed persistent anestrous to the end of the experiment. Five of the seven control rats also ceased cycling after seventeen to twenty-one days on the experiment. The two exceptions continued to show periodic estrous states, but with lengthened diestrous intervals.

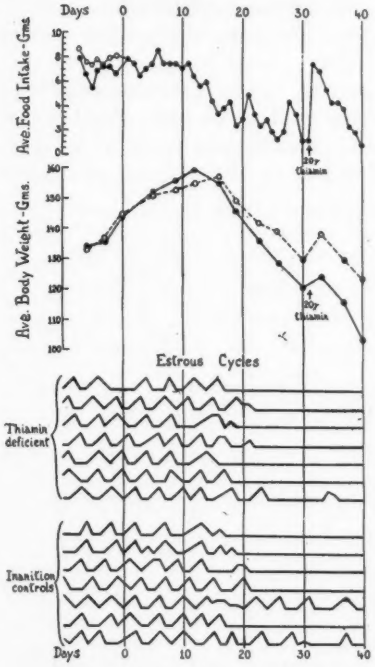

FIG. 1. Exp. I. Average daily food intake, weight curve and estrous cycles for thiamin-deficient rats (solid circles) and inanition controls (open circles).

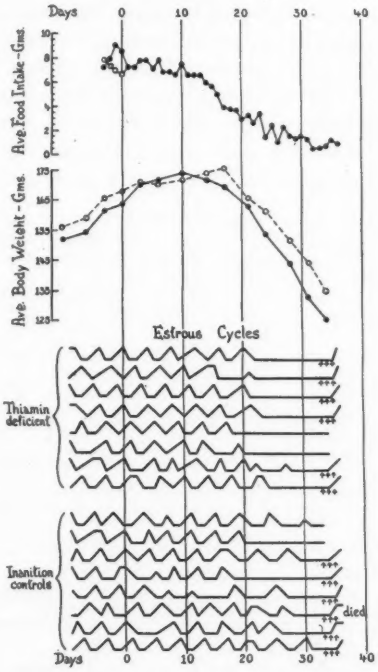

FIG. 2. Exp. II. Average daily food intake, weight curve and estrous cycles for thiamin deficient rats (solid circles) and inanition controls (open circles). Arrow indicates administration of 3 I.U. Follutein.

Experiment II. The two groups in this experiment contained eight rats each. The food intake and growth curves (Fig. 2) were essentially similar to those obtained in the first experiment. Estrous cycles ceased in the thiamin-deficient animals after eighteen to twenty-four days except in one animal which showed a slight stimulation (not complete estrous) on the twenty-seventh day. Six of the control animals exhibited continuous diestrous after eighteen to twenty-nine days.

In both groups certain of the animals received subcutaneous injections of chorionic gonadotropin (Follutein, 3 I.U. per day for three days) starting on the thirty-third day. All animals, whether inanition controls or thiamin deficient, responded to this substance by reaching

a state of complete estrous on either the thirty-fifth or thirty-sixth day (Fig. 2).

Organ weights and ovarian histology.

At the end of each experiment the thyroids, pituitaries, ovaries and adrenals of all animals were weighed. For comparison these organs were also removed from a group of seven normal female rats.

All of the thiamin-deficient and control animals lost considerable weight during the course of the experiment. Loss in body weight may be largely due to loss of reserve fat and general dehydration and does not necessarily imply a corresponding loss in the weights of organs

TABLE 1. EFFECT OF THIAMIN DEFICIENCY AND INANITION ON WEIGHTS OF ENDOCRINE GLANDS
(All organ wt./body wt. ratios calculated on the basis of initial body weight)

		No. animals	Avg. Body Wt. (gm.)		Thyroid		Pituitary		Adrenal		Ovary	
			Initial	Final	Avg. (mg.)	mg./100 gm. body wt.	Avg. (mg.)	mg./100 gm. body wt.	Avg. (mg.)	mg./100 gm. body wt.	Avg. (mg.)	mg./100 gm. body wt.
	Normals	7	149		9.66	6.48	8.63	5.79	19.99	13.42	25.09 ±2.13	16.85±1.13
Exp. I	Inanition controls	7	147	125	9.94	6.76	7.81	5.31	19.44	13.22	16.31 ±2.12	10.97±1.33
	Thiamin deficient	7	146	105	9.97	6.83	6.42	4.40	18.89	12.94	12.46 ±0.94	8.50±0.58
Exp. II	Inanition controls	7	168	135	11.32	6.74	7.95	4.73	19.64	11.69	30.51*±3.18 18.66‡	18.16 11.11
	Thiamin deficient	8	164	126	10.56	6.44	7.13	4.35	19.31	11.77	21.39†±1.19 16.61‡	13.04 10.12

* Average for five rats injected with Follutein.
† Average for six rats injected with Follutein.
‡ Average for two rats not injected with Follutein.

which have no stored materials. If the animals lost weight and the organ weight remained constant, calculation of organ weight on the basis of final body weight would result in an apparent increase in organ weight, or hypertrophy. Similarly, if both the animal and organ lost weight, calculation on the basis of final body weight would not reveal the atrophy of an organ unless the organ in question lost weight more rapidly than the animal. It therefore seemed more appropriate to consider the organ weights in relation to the initial body weights rather than the final weights. When the organ weights were calculated in this manner (Table 1) it was found that there were no significant differences between groups in the proportionate weights of the thyroid, pituitary and adrenal. The ovaries, however, showed marked changes.

In Experiment I both the thiamin-deficient and inanition control groups had ovaries significantly smaller than normal. Compared to the normal animals the t value for the thiamin-deficient group was 3.608 and for the inanition control group was 5.384, both representing very significant differences. The difference between the ovaries of the thiamin-deficient and inanition control groups was not significant.

In Experiment II, some of the animals received injections of Follutein for three days prior to sacrifice. The ovarian weights of these animals were not significantly different from the normal. However, the ovaries of the four animals which received no Follutein were smaller than normal and fell essentially within the range of those in Experiment I.

The pituitary apparently decreased slightly in actual weight, although in only one group was there a significant difference from the normal. This was the thiamin-deficient group of Experiment I, the group which suffered the greatest loss in body weight. However, when the pituitary weights were calculated on the basis of initial body weight, there were no significant differences among any of the groups.

Pituitary atrophy in under-fed animals has frequently been reported. The lack of effect in our experiments may be due to the fact that inanition was progressive and only became acute toward the end of the experiment, thus allowing insufficient time for a reflection in the pituitary weight. The same explanation may apply in the case of the adrenals, since other workers have found adrenal hypertrophy in response to inanition or thiamin-deficiency.

Histologically the ovaries of the thiamin deficient animals and the inanition controls showed little change from normal. In general there were fewer large follicles and more corpora atretica when compared with normal animals. There were also fewer follicles in stages of growth. The ovaries from those animals which had been injected with Follutein were indistinguishable from the normal gonads. Assuming that, prior to Follutein administration, these ovaries had been in a condition comparable to that in the ovaries of the non-treated animals, the normal state of the treated ovaries indicates an undiminished capacity for response to the gonadotropic substance.

DISCUSSION

The results show that the absence of thiamin in a diet otherwise adequate causes a progressive decrease in food intake resulting in loss of body weight and accompanied by cessation of estrous cycles. The same loss in body weight and cessation of cycles occurs even when thiamin is given if the total food intake is reduced to correspond with that of the thiamin-deficient rats. Thus the effect of thiamin deficiency on the ovary is not one of thiamin deficiency *per se*, but rather due to the resulting inanition.

The continued diestrous resulting from inanition apparently is not due essentially to ovarian failure nor to loss in sensitivity of the vaginal epithelium, since the injection of a gonad-stimulating substance (Follutein) brings about a prompt response manifested by the estrous type of vaginal smear, and the normal weight and histological character of the gonads. This ovarian response confirms previous studies by Parkes (1928), Marrion and Parkes (1929), Mulinos et al.

(1939), Werner (1939) and Stephens and Allen (1941). The absence of cycles must therefore be caused by failure of the pituitary to produce sufficient gonadotropic substance to stimulate estrogen production by the ovary. This pituitary failure apparently occurs as a result of inanition, whether the inanition is caused by a thiamin deficiency or by underfeeding. This is in accord with the reports of Mason and Wolfe (1930) and Werner (1939) who found that the gonadotropic potency of the rat pituitary was diminished during inanition.

SUMMARY

Normal female rats placed on a diet adequate in all respects except for the absence of thiamin, rapidly lost weight as a result of reduced food intake. The estrous cycles ceased within sixteen to twenty-four days. The ovaries were significantly smaller than normal although only slight histological changes were found. Another group of rats given the same diet, but in quantities comparable to those consumed by the thiamin-deficient rats, showed the same response in spite of adequate thiamin administration. The ovarian hypofunction and the resultant anestrous in the thiamin-deficient rats apparently were caused, not by the thiamin deficiency alone, but by the concomitant inanition.

In both the thiamin-deficient rats and their inanition controls the ovaries responded to administration of a gonadotropic substance, indicating that diminished gonadotropic function of the pituitary was the primary cause of the ovarian hypofunction and the persistent diestrous. The weights of the thyroid, pituitary and adrenal were not significantly altered by the experimental procedure.

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NOTES AND COMMENTS

STUDIES ON THE FAILURE OF DECIDUOMA FORMATION AFTER PLACENTATION IN THE MOUSE[1,2]

UTERINE sensitivity to deciduoma formation during pregnancy is limited to the time of implantation and formation of the placenta (Loeb, 1907, 1908; Courrier and Kehl, 1930; Allen, 1931). The desensitization of the uterus after placentation may serve to prevent superfetation, for as Weichert (1942) points out " . . . it seems possible that a second conception during pregnancy never occurs."

This period of uterine insensitivity continues in the pregnant lactating animal during early lactation. It is manifested by the delay of the implantation of the ova and results in a prolongation of gestation (Kirkham, 1916). The administration of estrogen to the pregnant lactating rat restores sensitivity to implantation (Krehbiel, 1941; Weichert, 1942). On the other hand, the results obtained by treatment with progesterone are varied. Weichert (1940) reports that progesterone may hasten implantation in the lactating rat, whereas Turpeinen (1943) finds that in the lactating mouse progesterone delays implantation of the ova. This divergence of results may be due to a species difference in reactivity to progesterone. In any case, the mechanism by which either estrogen or progesterone modifies uterine sensitivity during lactation is obscure, while the effects of these hormones on uterine sensitivity during pregnancy have not been investigated.

The following experiments were designed to determine whether estrogen or progesterone would overcome the desensitization of the uterus to deciduoma formation during pregnancy in the mouse.

EXPERIMENTAL

One oviduct was ligated in each of 30 young adult mice of the C_{57} and Swiss albino strains in order to effect unilateral sterilization of the animals. After mating, as determined by the presence of a seminal plug in the vagina, the mice were divided into four groups and—treatment was begun on the 9th day—as shown in Table 1. On the 10th day of pregnancy an attempt to produce deciduomata was made in all four groups by traumatizing the nonpregnant horn of the uterus with a suture placed longitudinally in the lumen. The hormone injections were continued during the subsequent 3 days, and the animals were sacrificed on the day following the last injection (14th day of pregnancy).

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[1] Aided by a grant, administered by Dr. Philip E. Smith, from the Rockefeller Foundation, New York.

[2] The progesterone used in this work was supplied through the courtesy of Ciba Pharmaceutical Products, Inc., and the estradiol benzoate by Schering Corporation.

In no case were deciduomata found in the traumatized non-pregnant uterine horn when examined at autopsy. The hormonal treatments had no deleterious effects on the pregnant horn, except in the animals receiving 0.5γ of estradiol benzoate daily. In this group approximately one-half of the foetal sites showed signs of resorption.

DISCUSSION

It has been demonstrated that progesterone is the only ovarian hormone needed in the castrated rat and mouse to sensitize the uterus for the formation of deciduomata (Rothchild, Meyer, and Spielman, 1940; Selye, 1940; Hooker, 1941) and that it is responsible for the maintenance of the reaction (Atkinson, 1944). Rothchild, Meyer, and Spielman (1940) have shown further that in the hormone-treated castrated rat the development of the deciduoma depends on the estrogen-progesterone ratio. The size of the

TABLE 1. EFFECT OF PROGESTERONE AND ESTROGEN ON THE FORMATION OF DECIDUOMATA IN THE PREGNANCY-DESENSITIZED MOUSE UTERUS

Group	No. of animals	Daily doses of		No. of animals developing deciduomata
		Progesterone (mg.)	Estradiol benzoate (γ)	
1	5	0	0	0
2	5	0	0.1	0
	5	0	0.5	0
3	5	1	0	0
	3	2	0	0
	2	5	0	0
4	5	1	0.1	0

deciduoma increases as the daily dose of progesterone is increased, or if the daily amount of progesterone is kept constant, the size of the deciduoma increases as small amounts of estrogen are given in addition to the progesterone. Above an optimal level, further increase of estrogen results in decrease in the size of the deciduoma and finally in complete inhibition of the reaction. These observations suggest that the desensitization of the uterus after the first trimester of gestation might be due to increased production of estrogen or decreased production of progesterone with advancing pregnancy. However, this theory does not seem to be substantiated by the present investigation which has shown that deciduomata fail to form in pregnant mice treated with as much as 5 mg. of progesterone daily. This amount of progesterone is 20 times the minimal effective dose necessary to sensitize the uterus in the castrated mouse (Hooker, 1941).

The present experiments have shown that estrogen, effective in the pregnant lactating animal (Krehbiel, 1941; Weichert, 1942), does not sensitize the uterus for the formation of deciduomata during pregnancy. This indicates that the desensitization of the uterus during gestation is not due to the same factors responsible for desensitization during lactation. As the factors involved during lactation are obscure, this observation does little to elucidate those at work during pregnancy. The only clue to the nature of the mecha-

nism responsible for the desensitization of the uterus during pregnancy is the report of Selye and McKeown (1935). These authors found that removal of the pregnant horn in the unilaterally pregnant rat permits the production of deciduomata in the remaining sterile horn. They concluded that the placentae are responsible for the inhibition of deciduoma formation in the intact pregnant animal. The mechanism by which the placentae might desensitize the uterus is not known, although it seems probable from the present work that it is not the result of a change in the estrogen-progestin balance.

SUMMARY

The uterus is insensitive to implantation and deciduoma formation after placentation and during early lactation. Unilaterally pregnant mice were treated with various doses of estradiol benzoate and progesterone and the non-pregnant uterine horn was traumatized. In all cases deciduomata failed to develop. Since estrogen restores uterine sensitivity during lactation, the present observations indicate that the desensitization of the uterus during pregnancy is due to factors other than those responsible for desensitization during lactation.

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ABSENCE OF POTENTIATION OF GONADOTROPIN AND STEROID FUNCTION IN MAMMALS BY COLCHICINE[1]

COLCHICINE has been found to increase the effect of frog pituitary in inducing ovulation *in vivo* and *in vitro* (McPhail and Wilbur, 1943). To ascertain whether a similar action might obtain in mammals the effects of colchicine administration have been examined under the following conditions: (1) ovulation in the rabbit produced by extract of pregnant mare's serum (PMS); (2) growth of the immature mouse ovary under the influence of PMS; and (3) the size of the prostate and seminal vesicles of castrated rats following injections of testosterone propionate.

OVULATION IN THE RABBIT

If colchicine should potentiate the action of PMS in the rabbit ovulation might occur earlier or the action could result in the rupture of a greater number of follicles, or both. Any augmentative action of the drug could presumably be demonstrated most easily near the liminal concentration of gonadotropin. In preliminary experiments a dose of 40 I.U. was found to approximate the threshold amount, causing ovulation in about half the animals. Pure-bred albino or chinchilla rabbits, paired in weight, were given 40 I.U. PMS intravenously. Colchicine in a dose of 1 mg./kg. was given to one subcutaneously, the other serving as control. Ten to twelve hours later laparotomy was performed on both; and at intervals of 2 to 3 hours the ovaries were examined to determine the time of ovulation and its extent.

Experiments carried out with liminal and slightly higher doses of PMS on 12 pairs of animals revealed only a slightly increased response in the colchicine-treated. The number of ovulation points totaled 31 for the colchicine-treated and 19 for animals receiving PMS alone. The number of large hemorrhagic follicles and probable rupture points was also slightly greater in the animals receiving both colchicine and PMS—23 as compared with 16. Ovulation of the colchicine-treated occurred earlier or in the absence of ovulation in the control ovaries in six pairs as compared with 2 cases in which the control animals ovulated earlier. In two pairs ovulation occurred at the same time and in two others ovulation failed to occur. None of the differences is statistically significant.

PMS AND OVARIAN WEIGHTS IN MICE

Using ovarian weights as a criterion the effect of colchicine administered with PMS gonadotropin was studied in mice. Fourteen animals 24 to 32 days old were injected daily for 5 days with 20 I.U. of PMS plus approximately 0.5 mg. colchicine daily per kilo body weight.[2] Thirteen litter mates were given the same quantity of PMS plus a volume of saline equivalent to the volume of colchicine injected. No increased action of gonadotropin was observed.

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[1] We wish to thank the National Research Council of Canada and the Eli Lilly Company for financial support; Dr. A. S. Cook of Ayerst, McKenna and Harrison for PMS and Mr. L. A. Casey of Schering Corporation for testosterone propionate.

[2] $LD_{50} \pm$S.E. mg./kg. for colchicine sample used was 2.596 ± 0.153 (data of Dr. K. K. Chen of Eli Lilly Company).

TESTOSTERONE AND THE WEIGHTS OF PROSTATE AND SEMINAL VESICLE GLANDS IN RATS

Colchicine was administered to rats along with testosterone propionate to determine whether the alkaloid would augment the action of the androgen in causing prostate gland and seminal vesicle hypertrophy. Twenty-four adult albino rats, castrated 10 days prior to treatment, were divided into three groups of 8 animals each. One group received subcutaneously 0.5 mg. testosterone propionate daily for 10 days. A second group was given the same amount of androgen plus approximately 0.14 mg. colchicine daily for the same period.[3] A third group received neither testosterone nor colchicine.

The average weight of prostates and seminal vesicles (2.22 gm.) for the group receiving colchicine and testosterone did not differ significantly from that for the group given testosterone alone (2.29 gm.). In both groups, however, the weights of the prostates and seminal vesicles were increased about 12-fold over those of the non-injected animals, the average weight for the glands in the latter being 0.19 mg. It was thought that the augmentative action of colchicine might not be demonstrable with the above large dose of androgen. Consequently the experiment was repeated using a much smaller amount on two groups of castrated rats.[3] Eight animals received 0.05 mg. of testosterone daily for 10 days and eight received the same amount of androgen plus 0.10 mg. of colchicine daily. Again the alkaloid was without effect; the average weight of the accessory sex organs was 1.36 gm. for the testosterone group and 1.45 for the group receiving testosterone and colchicine. The dose of colchicine was sufficiently large in both experiments to cause a statistically significant loss in weight in the injected animals.

SUMMARY

Colchicine was found to have no significant effect on the following reactions: (1) ovulation in the rabbit induced by PMS, (2) growth of the immature mouse ovary under the influence of PMS, and (3) the size of the prostate gland and seminal vesicles of castrated rats injected with testosterone propionate.

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[3] Colchicine injections omitted one day because of toxic symptoms.

NEW BOOKS

TEXTBOOK OF GYNECOLOGY. By Emil Novak, Associate in Gynecology, The Johns Hopkins Medical School. The Williams & Wilkins Co., Baltimore, Md. $8.00, Second Edition, 1944, 1–698 pp.

In the preface to the first edition of the book the author stated that it was unnecessary to make any apology for the combined title, Gynecology and Female Endocrinology, so intimarely associated are the two subjects. The title of the second edition is simply, "Textbook of Gynecology," the implication being that female endocrinology has already become an integral part of the practice of gynecology, which, in fact, it has for all well informed, progressive gynecologists.

This second edition contains forty-four chapters, sixteen of which are devoted primarily to the physiology of reproduction in the broadest sense. A very lucid discussion is given in these chapters of the physiology of menstruation, the anovulatory cycle, dysmenorrhea, amenorrhea, functional uterine bleeding, sterility, the menopause and related disorders. Even the serious student of the physiology of reproduction will find little that is inaccurate or misleading and he will be surprised that such a complicated and oftentimes confusing subject can be so carefully simplified as has been done in this book.

The chapters devoted to "straight" gynecology are equally well written and informative. The discussions of tumors, infectious processes, genital lacerations and displacements, and fistulae are well illustrated and the approach to the subject matter is such that the clinical aspects of the problems are always based on an accurate understanding of the pathological condition.

This book represents the experience of an expert pathologist and an experienced gynecologist who has for many years been one of the pioneers in applying to clinical practice the rapid advances which have been made in our knowledge of the physiology of the reproductive processes. No textbook of gynecology at present available is better written and none equals it in its discussion of the endocrine problems relating to gynecology.

WILLARD M. ALLEN

PROGRAM

OF THE

TWENTY SEVENTH ANNUAL MEETING

OF THE

ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

June 12 and 13, 1944
Chicago, Illinois

ABSTRACTS OF PAPERS

Papers Read

ENDOCRINE DISEASE AS ENCOUNTERED IN THE EXAMINATION OF 13 MILLION REGISTRANTS. *Rowntree, L. G.*, National Headquarters, Selective Service System, Washington, D. C.

No abstract.

THE ROLE OF THE LIVER IN THE METABOLISM OF SYNTHETIC ESTROGENS AND PROESTROGENS. *Segaloff, Albert* (introduced by Warren O. Nelson) Department of Anatomy, Wayne University, College of Medicine, Detroit, Michigan.

It is now generally acknowledged that the liver is a major site of inactivation for the natural estrogens. Very little such evidence is available for synthetic substances possessing estrogenic activity. Accordingly synthetic estrogens were studied by our technique of intrasplenic injection. It was found that diethylstilbestrol and hexestrol required 17 and 22 times as much, respectively, when injected into the spleen *in situ* as when injected into the transplanted spleen divorced from the portal circulation. Considerably smaller amounts of diethylstilbestrol dipropionate were required. This indicated that a considerable portion of the free compound was inactivated by the liver and that the inactivation probably proceeds through the hydroxyl groups since esterification affords protection against inactivation. In striking contrast to the behavior of the above compounds triphenylethylene, triphenylchlorethylene and 9,10-di-n-propyl-9,10-de-hydro-9,10-dihydroxy-1:2:5:6 dibenzanthracene required only one-eighth to one-fifth as much when injected into the spleen *in situ* as when injected into the spleen divorced from the portal circulation. It thus appears that the systemic activation proposed by Emmens for such proestrogens as the last three compounds takes place in the liver.

THE INFLUENCE OF HORMONES UPON THE CHEMIAL COMPOSITION OF THE BRAIN. *Weil, Arthur.* Northwestern University, Chicago, Illinois.

The paper is concerned with the effect of sex hormones, the adrenal gland and the hypophysis upon the phospholipid content of the brain of white rats. It had been shown previously that both hyper- and hypothyroidism do not change the phospholipid content qualitatively.—Removal of the gonads changes the different chemical constitution of the male and female brain to a more neutral intermediate level. Injections of homologous sex hormones (testosterone propionate and α-

estradiol benzoate) into immature rats have no qualitative effect upon the chemistry of the brain; however, injections of heterologous sex hormones change the male brain into a female one and vice versa. This effect may be obtained in both normal and castrated rats. Following adrenalectomy there is a considerable increase in the size of both male and female brains with a proportional increase in the amount of the different lipid fractions, but without qualitative changes. Hypophysectomy affects the male brain slightly more than the female brain and leads to a mild increase in the amount of phospholipids and their phosphorus content.

SUSTAINED REMISSION OF HYPERTHYROIDISM AFTER THIOURACIL THERAPY. *Astwood, E. B.* From the Medical Clinic of the Peter Bent Brigham Hospital and the Departments of Medicine and Pharmacology, Harvard Medical School, Boston, Massachusetts.

Shortly after hyperthyroidism had been controlled with thiouracil the withdrawal of medication in nine instances was followed by a return of symptoms and a rise in metabolic rate within 7 to 45 days. However, when treatment was not discontinued until metabolic equilibrium had been maintained with thiouracil for 6 to 9 months, continued remissions occurred. Of eleven such patients, ten have been observed closely for longer than three months and have exhibited no evidence of recurrence. In the event of subsequent relapse, treatment with a maintenance dose of thiouracil could be resumed as soon as symptoms are noted. Before thiouracil was withdrawn, all patients had been free of symptoms on a small maintenance dose and those with large goiters exhibited a decrease in the size of the gland and disappearance of the bruit. These signs were taken to imply that spontaneous improvement had begun.

METABOLIC EFFECTS OF THIOURACIL IN GRAVES' DISEASE. *Sloan, Margaret H.* and *Ephraim Shorr.* From the Russell Sage Institute of Pathology, The Department of Medicine, Cornell University Medical College and The New York Hospital, New York City.

A study was made of the effect of thiouracil on the disturbances in calcium, phosphorus, nitrogen, creatine-creatinine, cholesterol and 17-ketosteroid metabolism in patients with Graves' disease under carefully controlled conditions. The findings were correlated with other indices of thyroid activity. All these various metabolic derangements were corrected by the administration of thiouracil just just as occurs after subtotal thyroidectomy or in iodine remission. The remissions produced by this new drug are, therefore, physiologic.

EXPERIMENTAL AND CLINICAL STUDIES ON THE ACTION OF THIOUREA AND THIOURACIL. *Paschkis, K. E., A. Cantarow* and *A. E. Rakoff.* Endocrine Clinic, Jefferson Medical College and Hospital, Philadelphia, Pennsylvania.

The relative potency of thiourea and of thiouracil inhibiting enzyme systems *in vitro* was studied. In experiments on rats it was attempted to suppress overactivity of the anterior pituitary associated with the suppression of formation of thyroid hormone. The clinical effect of treatment of thyrotoxicosis with thiourea and thiouracil was studied in 20 cases. Establishment of a "permanent maintenance dosage" was attempted rather than intermittent treatment. Four cases in which surgery was contraindicated are of particular interest. Special attention was paid to the occurrence of toxic manifestations.

THE EFFECT OF THIOURACIL ON THE COLLECTION AND SECRETION OF RADIOACTIVE IODINE BY THE THYROID. *Larson, Roger, F. R. Keating, Jr., Rulon W. Rawson* and *Wendell Peacock.* From the Institute of Experimental Medicine, The Mayo Foundation, Graduate School, University of Minnesota (Doctor Larson), the Division of Medicine, The Mayo Clinic (Doctor Keating), Rochester, Minne-

sota, the Thyroid Clinic of the Massachusetts General Hospital (Doctor Rawson) and the Radiation Center, Massachusetts Institute of Technology (Doctor Peacock), Boston, Massachusetts.

An artificially radioactive isotope of iodine having a half-life of eight days was injected in chicks. The proportion of the substance collected by the thyroid and the rate at which that previously collected was lost from the thyroid were determined. The glands were digested in alkali and their content of radioactive iodine measured with a Geiger counter. The weight of the thyroid gland and the mean height of acinar cells were also studied. Varying doses of thiouracil injected or administered in the drinking water produced a striking decrease in the collection of radioactive iodine by the thyroid. This effect appeared within an hour after administration of thiouracil and had largely worn off in less than 24 hours. The drug did not alter the rate at which iodine previously stored in the gland was lost. Histologic changes did not appear until thiouracil had been administered for several days. The ability of thyroid glands made goitrous by prolonged administration of thiouracil to collect radioactive iodine also was diminished but this effect wore off rapidly when the drug was withdrawn.

THE ABSORPTION, DISTRIBUTION AND EXCRETION OF THIOURACIL AND THE IMPORTANCE OF THESE FACTORS IN THE TREATMENT OF THYROTOXICOSIS. *Williams, Robert H.* and *Howard M. Clute.* Thorndike Memorial Laboratory, The Boston City Hospital, Boston, Massachusetts.

Thiouracil is rapidly absorbed from the gastro-intestinal tract and is distributed throughout all of the body tissues and fluids. Tissues storing the largest amount are the pituitary, thyroid, adrenals and bone marrow; the skin, muscle and liver contain the smallest amount. Thiouracil in the blood is largely bound to protein, the cells possessing several times as much as the plasma. Most of the tissues of the body and also the gastro-intestinal contents possess the capacity to destroy thiouracil. About one-third of the ingested drug is excreted as such in the urine, but none in the feces. These observations, together with the clinical experience in the treatment of 105 thyrotoxic patients indicate that it is better to give frequent small doses throughout the day than one or two larger doses. If more than 0.4 gm. daily of the drug is administered, the concentration in the blood increases only slightly in proportion to the dosage. The concentration of thiouracil in the thyroid glands of 24 thyrotoxic patients showed no correlation with the clinical response of the patient, with the histological alterations in the gland, nor with its content of thyroxin iodine.

THE EFFECT OF THIOURACIL AND OF POTASSIUM THIOCYANATE ON THE COLLECTION OF RADIOACTIVE IODINE BY THE THYROIDS OF ANIMALS AND MAN. *Rawson, Rulon W., Ruth E. Cortell, Wendell Peacock* and *J. H. Means.* From the Thyroid Clinic of the Massachusetts General Hospital, Boston, Massachusetts and the Radioactivity Center Department of Physics, Massachusetts Institute of Technology, Cambridge, Massachusetts.

The effects of iodine-inhibited, and iodine-resistant goitrogens on the thyroid's collection of iodine have been determined. Experimental goiters produced by potassium thiocyanate, an iodine-inhibited goitrogen, were found to collect 153% more of the administered radioactive iodine than did the thyroids of control animals. Goiters produced by the administration of thiouracil, an iodine-resistant goitrogen, though extremely hyperplastic, collected less than 18% as much of the administered iodine than did the thyroids of control animals. In a similar series of experiments the avidity for iodine of rat thyroids made goitrous by thiouracil two, five, ten and twenty days after stopping the administration of the drug was determined to learn the rate of recovery from the block produced by this agent. Since

thiouracil goiters can be prevented by thyroxine, the thyroid's avidity for iodine was determined in rats treated with thiouracil and thyroxine. Thyrotoxic patients following preparation for thyroidectomy with thiouracil were found to excrete in the urine most of the administered radio-active iodine. Very little of the iodine was demonstrated in the thyroids removed at operation. Patients made goitrous by potassium thiocyanate administered in treatment of hypertension excreted very little of the administered iodine.

ESTROGEN ACTIVITY IN THE EVISCERATED RAT. *Szego, Clara M., Sidney Roberts,* and *Leo T. Samuels.* Division of General Physiology and Physiological Chemistry, Department of Physiology, University of Minnesota Medical School, Minneapolis, Minnesota.

Doses of estrone and α-estradiol sufficient to cause maximal response in three-month-old female rats two weeks after ovariectomy were ineffective in similarly-prepared eviscerated animals (kidneys intact). The index of estrogen activity employed was the increase in uterine water content. Responses to α-estradiol were noted in control animals autopsied ten hours after administration of 0.2γ/100 gm. body weight subcutaneously in sesame oil, five hours after 0.2γ intravenously in saline, and six hours after 0.5γ injected into the cervical lumen. No response was obtained in eviscerates with 0.2–0.5γ α-estradiol or estrone over periods varying from three to twenty-four hours after the first of divided dosages by combined routes. Large doses of intravenously-injected α-estradiol were inactive in eviscerated rats previously maintained on a diet containing 1% desiccated thyroid, even though the basal metabolic rates five hours after evisceration were in the normal range. Non-eviscerated, thyroid-treated animals (B.M.R., +50%) responded normally. These results indicated that removal of the viscera interfered with uterine response to estrogen. Additional experiments have been carried out employing gut removal alone. Moreover, evisceration has been performed at short intervals *after* estrogen administration.

EFFECTS OF EXOGENOUS SEX HORMONES ON THE GONADS OF SPARROWS. *Pfeiffer, Carroll A.* Department of Anatomy, Yale University School of Medicine, New Haven, Conn.

Administration of 0.25 mg. of testosterone propionate daily to adult male sparrows at the beginning of the breeding season markedly increased the size of the testes within two weeks and maintained maximum development for at least 80 days. If treatment was stopped after two weeks the testes regressed to their former size or smaller within a week but could be enlarged again, although not as markedly. Eventually they regressed to the non-breeding condition despite continued treatment. When the same dose of androgen was given to young juvenal birds whose testes were readily stimulated by gonadotrophins but whose pituitaries were unresponsive, enlargement of the testis did not occur. Androgen stimulated, although to a less degree, the ovaries of mature but not juvenal females. In both types of females the oviducts became enlarged, but less so in juvenals, indicating that the greater development in adult females was due to estrogen released by the stimulated ovaries, since at either age the oviducts had about the same sensitivity to injected estrogen. Estrogens inhibited the normal enlargement of both testes and ovaries. Either hormone injected in oil slightly inhibited PMS-induced testicular enlargement, presumably because the unabsorbed oil interfered with absorption of the PMS.

DEPENDENCE OF OESTROUS CYCLES IN RATS ON VITAMIN D. *Richter, Curt P.* and *Katherine K. Rice.* Psychobiological Laboratory, Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore, Maryland.

In preliminary studies on small numbers of rats it was found that female rats given access to only yellow corn and water for several months were inactive and underweight, had poor appetites and remained in a constant dioestrous condition.

When given access to cod-liver oil, or to crystalline vitamin D_3 in cotton-seed oil, or exposed to ultraviolet rays, the deficiency was corrected almost at once. Within a day they became more active, and had increased appetites, and within 4 days cornified cells appeared in the vaginal smears. Within 15 days the animals often showed a full return to all normal levels. These observations have now been confirmed on larger groups of rats with histological sections of ovaries and uteri and also experiments on reproductive activity. These experiments demonstrate the close dependence of the reproductive function on vitamin D.

A Urine Color Reaction for Human Pregnancy. *Guterman, Henry S.* (introduced by Samuel Soskin). From the Department of Metabolism and Endocrinology, Michael Reese Hospital, Chicago, Illinois.

Pregnandiol excretion begins at approximately the time of ovulation, increases through the second half of the menstrual cycle, and ceases prior to the onset of the flow unless fertilization has occurred. In the latter event pregnandiol is excreted in increasing amounts as normal pregnancy progresses. Based upon these facts, a simple chemical procedure for the diagnosis of pregnancy has been devised. By modifying Astwood's and Jones' (J. Biol. Chem., *137:* 397, 1941) method for the extraction of pregnandiol from urine and Talbot's (J. Clin. Endocrinol., *1:* 668, 1941) colorimetric determination of the substance, it was possible to obtain a qualitative color reaction which agrees with the results of the Friedman rabbit test. One hundred cc. of the first morning specimen are used and the test is completed in three hours. Several tests can be carried out simultaneously. This procedure has the following advantages: (1) rapidity (3 hours); (2) does not involve use of animals; (3) becomes positive earlier than the Friedman test; (4) in conditions other than normal pregnancy, in which the prolan excretion is raised sufficiently to give a positive Friedman reaction, the pregnandiol test is negative.

Menorrhagia—Its Control by Prolactin. *Kupperman, Herbert S., Paul Fried* and *L. Q. Hair.* Department of Experimental Medicine, University of Georgia School of Medicine, Augusta, Georgia.

Menorrhagia, either functional or organic, was favorably influenced by administration of lactogenic preparations (pituitary). Menorrhagia associated with uterine fibroids, cystic ovaries or chronic pelvic inflammatory disease was suppressed by administration of prolactin in the majority of patients. Those patients, however, in the latter two cateogires, were more resistant to therapy. Normal cyclic bleeding followed cessation of prolactin therapy in many of the patients receiving one or more courses of treatment. This permanence, or semi-permanence, in the effect of prolactin afforded a sufficient interval to enable the patient to undergo a more strenuous régime to correct the underlying cause of the excessive uterine bleeding. Some success was obtained in mitigating dysmenorrhea by prolactin therapy when it was associated with excessive uterine bleeding. In addition, the signs and symptoms of threatened abortion were alleviated by administering prolactin to 3 of 6 patients with histories of previous abortions. Administration of lactogenic preparations to normally cyclic women had no appreciable effect upon the length of cycle, menstrual bleeding or morphological appearance of the endometrium. The effectiveness of lactogenic hormone in controlling uterine bleeding is probably linked up with its uteotrophic properties. In view of the innocuous effect of prolactin upon the normal menstrual cycle and corrective action in metropathic menorrhagia, its use is advocated in the control of excessive uterine bleeding.

The Vaginal Smear in the Diagnosis of Cancer of the Uterus. *Fremont-Smith, Maurice.* Vincent Memorial Laboratory, Mass. General Hospital. Boston, Massachusetts.

Vaginal smears from approximately 800 cases have been examined. Of these,

634 smears were negative, 117 positive for cancer (cervix or body) and 8 suspicious (no diagnosis possible). In the case of 6 positive smears a fixed tissue diagnosis of cancer has not been established (unfinished cases). 22 diagnoses are listed as mistakes (positive smear with negative fixed tissue diagnosis, or negative smear in cases proven to have cancer). In 3 cases cervical carcinoma *in situ* was diagnosed by vaginal smear. Cervical biopsy in one of these two was indeterminate; cancer was found by section. 6 unsuspected cancers of the cervix were diagnosed by vaginal smear, and confirmed by fixed tissue section. The technique of collection and staining is described.

PERLINGUAL ABSORPTION OF PROGESTERONE AND ANHYDROHYDROXYPROGESTERONE. *Greenblatt, Robert B.* Southeastern Medical Center, Oatland Island, Savannah, Georgia.

Proof for the sublingual absorption of such steroids as estradiol, methyl testosterone or anhydrohydroxyprogesterone has not been convincing inasmuch as part of these preparations may be swallowed. On the other hand, progesterone is inactivated by ingestion. If it can be shown that progesterone is effective when applied sublingually, it would afford proof that the other steroids are also absorbed by way of the oral mucosa. Observations were made on 62 patients following 133 courses of therapy. Sublingually anhydrohydroxyprogesterone was used in 92 courses and progesterone in 41 courses. Endometrial biopsies were taken when feasible prior to and after administration of a course of therapy. The criteria for effective absorption of each preparation were: the induction of "progesterone withdrawal bleeding" in amenorrheic patients; the effective arrest of menorrhagia six to ten days after withdrawal of the drug, i.e., "arrest of bleeding according to plan." The effectual absorption of these preparations was surmised from the beneficial effects noted in patients with mastodynia, premenstrual tension, certain patients with dysmenorrhea, as well as its varying effect on sexual libido. Occasionally in lieu of the withdrawal bleeding that was expected following sublingual medication, the patients experienced molimina, breast turgidity or uterine cramps.

THE EXCRETION OF SODIUM PREGNANEDIOL GLUCURONIDATE IN THE URINE FOLLOWING THE ORAL ADMINISTRATION OF ANHYDROHYDROXYPROGESTERONE AND PROGESTERONE TO PATIENTS WITH SECONDARY AMENORRHEA. *Allen, Willard M., Ellenmae Viergiver,* and *Samuel D. Soule,* Washington University School of Medicine, St. Louis, Missouri.

Anhydrohydroxy-progesterone or progesterone was given orally in doses from 200 to 500 mg. per day for a period of from one to six days to several patients with secondary amenorrhea. The urine was collected daily under butyl alcohol for a period of two to three days before therapy, during therapy, and for a period of from four to seven days following cessation of therapy. The urine was then extracted with butyl alcohol and the sodium pregnanediol glucuronidate determined by the method of Viergiver and Allen. In all cases there was a considerable increase in the amount of sodium pregnanediol glucuronidate excreted in the urine while the preparations were being taken orally. The amount recovered in the urine varied from six to twelve per cent of the theoretical amount. The excretion of ketosteroids was also determined but in no case was there any indication that an increased amount of ketosteroid was excreted while the two preparations were being taken orally. Estrogens were likewise studied in the urine by a modification of the Kober method but no estrogen was measurable by this method either before, during or after therapy.

OBSERVATIONS ON THE EXPERIMENTAL USE OF GONADOTROPIC EXTRACTS IN THE HUMAN FEMALE. *Davis, M. Edward* and *Arthur Hellbaum.* From the Department of Obstetrics and Gynecology, the University of Chicago, and the Department of Pharmacology, the University of Oklahoma, Oklahoma.

During the past year gonadotropic extracts have been made available for experimental use in man. These extracts have been brought to a high degree of purity by the elimination of much of the extraneous substances. Fractionated extracts have been found to contain F.S.H. only, whereas unfractionated extracts contain L.H. as well as F.S.H. The efficacy of these pituitary extracts in gonadal stimulation and the production of ovulation has been demonstrated in the rat, the sheep, and the cow. These extracts were tested for reactions when administered intramuscularly and intravenously. Earlier samples produced some undesirable symptoms but these have become less common. Considerable evidence has been accumulated previously concerning the histologic appearance of the ovary during the immediate postpartum period. The absence of normal follicle activity suggested this period in the life cycle of the ovary as ideal for the study of gonadotropin effects. The changes induced by these pituitary preparations on the human ovary in the immediate postpartum period were studied. In most patients an ovary was removed for histologic study. Follicle growth of physiologic and pathologic proportions was induced. It was not possible to produce ovulation although it was felt that this goal can be achieved by continued observations. It is possible that the human ovary in the immediate postpartum period is not normally receptive to gonadotropic stimulation.

THE CAUSES, DIAGNOSIS AND TREATMENT OF VARIOUS TYPES OF SEXUAL INFANTILISM IN FEMALES. *Wilkins, Lawson.* Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland.

Sexual infantilism in females may be due to: (A) primary ovarian deficiency; (B) ovarian deficiency secondary to a lack of secretion of pituitary gonadotropin. In the first group are cases due to (1) ovarian agenesis; (2) destruction or removal of the ovaries before puberty. In the second are cases of (1) pituitary dwarfism; (2) Froehlich's syndrome; (3) specific deficiency of pituitary gonadotropin. The clinical findings in these various conditions are illustrated and discussed. The value of diagnostic studies, including the assay of F.S.H. in the urine and the 17-ketosteroids is considered. The importance of exploratory laparotomy or peritoneoscopy is stressed. Indications for various types of treatment are discussed and the shortcomings of gonadotropic therapy are pointed out. Plans of treatment with estrogen and progestin and with androgen in proper cases are described. A case is reported of a patient with pituitary infantilism who was successfully treated.

OVARIAN FOLLICLES IN NORMALLY CYCLIC AND PERSISTENT-ESTRUS RATS. *Jeffers, Katherine R.* Duke University, Durham, North Carolina.

Counts and measurements were made of growing and degenerating follicles in ovaries of control rats and rats which had been in estrus for prolonged periods of time. The sectioned ovaries were from animals of the DA strain. The controls were 1) rats in estrus with enlarged follicles prior to ovulation; 2) rats in estrus with recently formed corpora lutea; and 3) pregnant (20 days) rats with large functional corpora lutea. It was found that the average number of follicles per ovary increases after ovulation, with growth taking place in all sizes of follicles up to 500 microns in diameter. The increase is most marked in early stages of growth. An increase in follicles 200–500 microns in diameter occurs in late pregnancy. Degeneration of follicles is more marked after ovulation. Two groups of animals with persistent estrus were studied: one nonpregnant and the other pregnant. The average number of follicles in the persistent-estrus animals is less than half that of the controls. Growth at all stages is slower, apparently, although very large vesicular follicles (up to 800 microns in diameter) occur in the non-pregnant rats.

RELATION BETWEEN LIGHT-DARK RHYTHMS AND HOUR OF LAY OF EGGS EXPERIMENTALLY RETAINED IN THE HEN. *Rothchild, Irving,* and *R. M. Fraps,* Bureau of Animal Industry, U. S. Dept. of Agriculture, Beltsville, Maryland.

The present authors have demonstrated that removal of the avian ruptured ovarian follicle results in retention of the oviducal egg for 1 to 7 days beyond expected time of lay (in press). We have since found that removal of all follicles in the phase of rapid growth together with the ruptured follicle results generally in more uniform retention of eggs (10–28 hours beyond expected time of lay). Further, most such retained eggs were laid during normally lighted hours (6:00 A.M.–8:00 P.M.), the peak of the distribution curve falling between 1:00 and 3:00 P.M., or 7 to 9 hours following onset of light. In similarly operated birds placed under reversed lighting conditions (lights 4:00 P.M.–6:00 A.M.) immediately following operation, the peak in the distribution curve for hour of lay fell between 11:00 P.M. and 2:00 A.M., or 7 to 11 hours following onset of light. Most eggs were retained either 10–18 or 28–36 hours, thus being laid during either of two successive lighted periods. Since the hour of (delayed) lay in these birds conforms to alternations in light and darkness in the complete absence of the normal regulating mechanism, the participation of an extra-ovarian agency, possibly the pituitary, seems indicated in the process of avian oviposition.

THE ROLE OF THE PITUITARY IN TOXIC GOITER. *Thompson, Willard O.* and *Phebe K. Thompson.* University of Illinois College of Medicine, Chicago, Illinois.

Ever since the demonstration of the thyrotropic activity of the pituitary there has been great interest in its role in toxic goiter. During the past several years we have observed the effect of irradiation of the pituitary in 38 patients with toxic goiter. In 7 of them the metabolism dropped permanently to within normal limits. In 16 a temporary reduction in metabolism of from 15 to 52 points was observed. In 15 patients there was no change. These observations appear to support the hypothesis that in some patients with toxic goiter the anterior lobe of the pituitary stimulates the thyroid to overactivity. While the results of treatment with thiourea, thiouracil and radioactive iodine are very important, these materials do not appear to attack the underlying cause of the disease.

GROWTH AND TREATMENT OF DWARFS AND GIANTS. *Bayer, Leona M.* Stanford University School of Medicine, San Francisco, California.

Sixteen presumably hypopituitary dwarfs and four hyperpituitary giants were followed from one to 13 years through periods of treatment and control. Data are analyzed particularly from the longitudinal viewpoint. Growth of dwarfs and giants shows contrasts similar to differences between early-maturing and late-maturing normals. In *growth rate* dwarfs tend to late adolescent spurts, sometimes of considerable magnitude; giants to early spurts. In *stature* and *skeletal development* dwarfs fall further behind normal before adolescence, recover gradually thereafter. Inversely, giants show preadolescent acceleration, with subsequent return toward normal. When *sitting height-stature ratios* are noted, dwarfs tend to be short legged; giants may be long legged before adolescence, short or medium legged afterwards. *Basal metabolic rates* have a surprising distribution; many dwarfs have rates above normal; giants below normal. Contrastingly, *intelligence quotients* tend to be low in dwarfs, high in giants. Observations include periods of treatment with thyroid, growth hormone, sex hormones, and their combinations. During adolescence the inherent growth tendency often supersedes effects of any treatment. In dwarfs, thyroid periods most consistently show stimulation of all phases of growth. With other extracts, especially sex hormones, a few striking improvements encourage continued trial.

HYPOPITUITARISM ASSOCIATED WITH EPILEPSY FOLLOWING HEAD INJURY: REPORT OF TWO CASES. *Lerman, Jacob* and *James H. Means.* Massachusetts General Hospital, Boston, Massachusetts.

Two patients with hypopituitarism and epilepsy were encountered. The first, a male of 46, was shot through the head at the age of 15. The bullet entered the left

orbit, traversed the pituitary fossa and lodged behind the posterior clinoids. Growth and development ceased and gradually epileptiform seizures supervened. The second, a male of 48, was injured in World War I by the explosion of a shell. He received several shrapnel wounds and was knocked unconscious. He, also, developed epileptic seizures. They both presented characteristic clinical and laboratory evidence of hypopituitarism. They differed only with respect to stature. The first patient was a partial dwarf; the second, having received his injury at the age of 22, was of normal size. Diagnosis and specific therapy are discussed.

CRETINOUS PITUITARISM. *Dunn, Charles W.* Philadelphia, Pennsylvania.

Myxedema can result from primary deficiency of pituitary thyrotropic secretion. The condition can develop in infancy and resembles clinically infantile myxedema due to primary fetal thyroid deficiency. The latter is characterized by retarded fetal development. Five cases of cretinous pituitarism are reported in all of which earlier diagnosis of thyrogenic cretinism had been made. Thyroid treatment had evoked toxic symptoms. Mental advancement disproportionate to administered thyroid had occurred but statural and sexual development had halted at from 10 to 14 years. The treatment should be addressed to the pituitary factor in the disorder.

ON THE RENOTROPIC ACTION OF AN ANTERIOR PITUITARY EXTRACT. *Selye, Hans* and *Kai Nielsen.* Department of Anatomy, McGill University, Montreal, Canada.

Previous experiments showed that various steroids stimulate the growth of kidney tissue. Since extracts of the anterior lobe of the hypophysis also cause renal enlargement, it was felt that the histological changes caused by anterior pituitary extracts should be compared with those elicited by steroids. Unilaterally nephrectomized female rats, having an initial average weight of 55 gm., were divided into two groups. Group I received no further treatment. Group II received two daily subcutaneous injections of a crude alkaline beef pituitary extract. After two months of treatment, when all animals were killed, the average body weight of the treated rats was 205 gm. and the average kidney weight 2150 mg. In the control group the corresponding average weights were 157 gm. and 1222 mg. respectively. It appears that the compensatory hypertrophy of the kidney remnant is more intensely stimulated by the extract than could be accounted for by its body-growth-promoting action. Histologically both the glomeruli and the tubules of the kidneys were enlarged but otherwise apparently normal. The liver weight was also markedly increased by this extract (14.3 gm. in the treated, 7.7 gm. in the controls). It is concluded that certain anterior lobe extracts selectively stimulate renal and hepatic growth. In the mouse, combined administration of anterior lobe extract and testosterone enlarged the kidney up to 5 times its normal weight.

DO MASSIVE DOSES OF ESTROGEN DEPRESS THE HUMAN PITUITARY AND THUS THE OVARY? *Abarbanel, A. R.* Department of Obstetrics and Gynecology, George Washington University School of Medicine, Washington, D. C.

A series of patients with excessive functional meno-metrorrhagia bleeding from endometria showing cystic glandular hyperplasia were given from 600 to 1200 mg. of oral diethystilbestrol over a period of 6–12 weeks. Endometrial biopsies at weekly or bi-weekly intervals disclosed a marked proliferative effect during the course of therapy. Within 5–10 days after the estrogen was stopped, estrogen-deprivation bleeding ensued. In each case biopsies taken 3–4 weeks later showed a strong secretory pattern and in several instances, a marked decidual reaction. In 10 of 12 such cases, menses became rhythmically cyclic and normal in amount, while follow-up premenstrual endometrial biopsies revealed a secretory pattern. These results are in harmony with the cytological studies of A. Sevringhaus, who

postulated that estrogen in small doses stimulated increased functional activity of the basophiles of the anterior pituitary (increase in mitochondria and Golgi apparatus and degranulation). Larger doses stimulated, in addition, the eosinophiles of the anterior hypophysis in a similar manner, releasing the luteinizing fraction. The evidence presented by these studies tends to confirm the second part of A. Sevringhaus' postulates. Further, it is in keeping with the experimental evidence of Hohlweg and Westman, in inducing ovulation and/or luteinization in young female rats with large doses of estrogen over a short perilod of time. Moreover, the failure of large doses of estrogen seemingly to depress the anterior pituitary is in line with the author's previous reports on the effect of estrogen on lactation in the human.

CHANGES IN THE RESPIRATORY QUOTIENT ASSOCIATED WITH DEVELOPMENT OF EXPERIMENTALLY PRODUCED OBESITY IN THE MONKEY (MACACA MULATTA). *Brooks, Chandler McC.* and *E. M. Bridge.* Departments of Physiology and Pediatrics, Johns Hopkins Univ. School of Medicine, Baltimore, Maryland.

Experimentally-produced lesions involving the suprachiasmatic areas and the tuberal region of the hypothalamus cause obesity in monkeys (Brooks, Lambert and Bard, 1942). Metabolic studies were made on three monkeys before and after such an operation, on a fourth during the static phase of obesity, and on a fifth normal monkey. One monkey was fed an excess of food after operation, one was given the same amount received before operation and one was restricted to its daily pre-operative intake. In this latter case the food was divided into four equal parts which were given at 9:00 A.M., 12:00 P.M., 3:00 P.M. and 6:00 P.M. The basal respiratory quotient was found to be somewhat above normal during the post-operative period of rapid weight gain. Furthermore, ingestion of food produced a rise in R. Q. (.81–.85 to 1.04–1.12 uncorrected) far exceeding that which occurred, after ingestion of similar quantities of food, in the normal control monkey and in these same animals before operation. In the monkey which had long been obese and in the three recently prepared animals after they had attained a static phase of obesity, the change in R. Q. after feeding was less marked (.82–.85 to .91–.95 uncorrected). The duration of R. Q. changes appeared to be related to the quantity of food ingested. The hypothesis suggested by these results is that, in the monkey, following this type of hypothalamic lesion a high proportion of food ingested is converted to fat. After obesity has been fully attained changes occur which diminish this tendency to convert ingested food to fat.

IS OBESITY ENDOCRINE OR WHY FAT PEOPLE EAT. *Richardson, Henry B.* From the Department of Medicine, the New York Hospital, and Cornell University Medical College, New York City.

Endocrinology in the popular view is the modern magic, and is supposed to supply both the explanation and the cure of obesity. Contrary to popular view not all endocrine cases are obese nor do all fat people have endocrine disturbances. In the great majority of cases no endocrine defect can be demonstrated and no consistent disturbances in the basal metabolism, sugar tolerance or specific dynamic action have been observed. It is not known whether the obese person lays down fat with any more facility than the normal or has any more difficulty in oxidizing it. An important factor is motivation in eating and this is extremely varied. The fundamental understanding of the motives requires a knowledge of the life history as to the family during childhood, the development of maturity, the adult situation and the personality reactions. With these data it is possible to understand the motivations in eating and being fat, not only the expressed motivations, but some of the symbolic meaning and psychological gain of the obesity. These observations throw more light on the etiology of obesity than all the present laboratory tests. The suc-

cessful reduction of weight depends on the degree to which the psychological factors are understood by the patient or utilized in the treatment by the doctor.

CYTOLOGICAL CHANGES IN THE PARATHYROID GLANDS FOLLOWING BILATERAL NEPHRECTOMY OR LIGATION OF THE URETERS. *Baker, Burton L.* Department of Anatomy, University of Michigan, Ann Arbor, Michigan.

The parathyroid glands are known to enlarge as a result of renal insufficiency and it has been suggested that in this condition, they are functionally hyperactive. It was thought that bilateral nephrectomy or ureteral ligation might alter the structure of the parathyroid cells and thus aid in the elucidation of their cytology. One of these operations was performed on 23 adult rats with 12 litter-mates serving as controls. This report is concerned primarily with changes observed in glands prepared by the Nassonov-Kolatschev technique. The cells of the normal rat parathyroid prepared by this method vary considerably in size, density of cytoplasm and form of the Golgi apparatus. The operative procedures employed caused the appearance of many large cells whose Golgi apparatus was located on only one side of the nucleus. It tended to possess a more spherical contour than in the normal glands and was definitely enlarged. Dark osmiophilic cells, which have been reported to be more numerous in rats subjected to low calcium and low phosphorus diets, were not significantly increased in number. The changes observed were independent of sex; they were similar after both types of operation and, hence, not dependent on the presence of renal tissue.

ACTION OF ESTROGEN ON PLASMA CALCIUM AND ENDOSTEAL BONE FORMATION IN PARATHYROIDECTOMIZED PIGEONS. *Riddle, Oscar* and *Vita M. Rauch.* Carnegie Institution, Department of Genetics, Cold Spring Harbor, New York.

In the race (Carneau) chiefly used, the four parathyroids are normally separated completely from the thyroids; parathyroidectomy, as performed here by G. C. Smith, avoids injury to thyroids. In operated birds of both sexes plasma calcium quickly decreased from approximately 9.0 to 6.1 mg. % (19 females) or 6.0 mg. % (20 males). In young birds (2–4 mo.) estradiol benzoate, 0.5 mg. daily for 4 days, increased plasma calcium to 26.0 mg. in 16 normal and to 20.9 mg. in 18 parathyroidectomized birds; macroscopically the femurs of both groups showed no more than traces (grade 1) of transformation of marrow to bone. Six older (4 + mo.) males gave similar results; in one of six older operated females the entire marrow cavity was replaced by bone (grade 4). Dosage over 15 days, 0.25 mg. daily, produced rather similar increases of plasma calcium in normal (to 25.0 mg. for 18) and parathyroidectomized birds (to 19.4 mg. for 12). Ossification of grades 2 and 3 predominated in younger birds, grades 3 and 4 in older birds, both operated and intact. The more characteristic actions of estrogens on blood calcium and bone in pigeons occur in the absence of the parathyroids.

TENSILE STRENGTH OF TISSUES AS INFLUENCED BY MALE SEX HORMONE. *Herrick, Earl H.* Elizabeth Clay Howald Fellow, Ohio State University, Columbus, Ohio.

Recognizing that young fowls treated with male sex hormone (testosterone propionate) developed skin that was more than normally resistant to tearing, measurements were made to determine the degree of this condition. Apparatus was designed to measure the breaking strength of selected tissues. Samples of skin from young fowls treated with 50 mg. of male sex hormone showed nearly 100 per cent greater tensile strength than like samples from untreated birds. The gastrocnemius muscle from the leg broke at 41 per cent greater pull in the treated than in normal fowl. The total wing muscles from the upper part of the wing were 45 per cent stronger in the treated than untreated birds. Histological conditions were studied.

THE HORMONAL REGULATION OF THE THERMO-REGULATORY FUNCTION OF THE SCROTUM. *Andrews, Frederick N.* Purdue University Agricultural Experiment Station, Lafayette, Ind.

Experiments carried out by the author and coworkers have shown that in the rat, ram, bull and boar there is a relationship between the development of the testis and the production of spermatozoa and the ability of the scrotum to respond, by contraction and relaxation, to changes in environmental temperature. When these species of animals were castrated prior to sexual maturity the scrotum failed to develop its thermo-regulatory function and if gonadectomy was performed after sexual maturity the thermo-regulatory function was lost. Studies on 64 sexually mature male albino rats showed that significant decreases in seminal-vesicle and scrotal-sac weights, and in the height of scrotal contraction occurred during the first, second and fourth weeks after castration, respectively. Coefficients of correlation of 0.915 between scrotal-contraction height and scrotal-sac weight, of 0.801 between scrotal-contraction height and seminal-vesicle weight, and of 0.843 between weights of the scrotal sacs and seminal vesicles were demonstrated. Fifty male rats were castrated at 35 days of age and injected with 0.5 mg. of testosterone propionate daily for periods of from 7 to 21 days beginning on the 90th day of age. A second group of 50 rats was castrated at 90 days of age and injected immediately with 0.5 mg. of testosterone propionate daily for from 7 to 21 days. Significant increases in height of scrotal contraction and weights of the scrotal sacs and seminal vesicles were obtained in rats castrated at 35 days of age and subsequently injected with testosterone. The injection of testosterone into sexually mature rats following castration on the 90th day of age adequately maintained scrotal sac weight and significantly increased seminal vesicle weight and the contractility of the scrotum. It is concluded that the development and maintenance of the thermo-regulatory function of the scrotum is dependent upon the testicular hormone.

THE EFFECT OF CASTRATION AND TESTOSTERONE PROPIONATE ADMINISTRATION ON THE PHOSPHORUS METABOLISM OF THE PELVES AND FEMURS OF THE MOUSE AS DETERMINED BY RADIOACTIVE PHOSPHORUS STUDIES. *Fox, R. P.* and *C. D. Kochakian.* Department of Vital Economics, University of Rochester, Rochester, New York.

Mice of the Murray-Little dba strain were divided into four groups: a) normal, b) castrate, c) normal implanted with pellet of testosterone propionate, d) castrate implanted with pellet of testosterone propionate. They were castrated and implanted at one month of age. Animals from each group were decapitated after 5, 7, 9 months. Twenty-four hours before autopsy radioactive phosphorus was injected intraperitoneally as the disodium phosphate. After fat extraction and glycol ashing, the uptake of P^{32} by the femurs and pelves was measured in the usual manner. The femurs and pelves of the castrated contained more radioactive phosphorus than those of the normal mice, whereas the femurs of the treated showed values similar to those of the normal mice. These effects were found at 5, 7 and 9 months. The femurs and pelves of the castrated mice were longer than those of the normals, while the latter, in turn, were longer than those of the treated animals. There was no difference in either ash content or calcium and phosphorus content as a result of castration or hormone administration.

METABOLISM OF TESTOSTERONE BY LIVER AND OTHER TISSUES IN VITRO. *Samuels, Leo T.* From the Division of Physiological Chemistry. Department of Physiology, University of Minnesota Medical School, Minneapolis, Minnesota.

The metabolism of testosterone when incubated with minces of liver and other tissues has been studied, using the colorimetric reaction of Koenig, *et al.* (1941). Spectrophotometric methods have been used to identify the products resulting

from alterations in the testosterone molecule. Testosterone is destroyed by normal adult rat liver mince at an average rate of 0.125 mg. per gm. tissue per hour. An atmosphere of nitrogen, or the presence of either 0.05M sodium fluoride or 0.01 M sodium cyanide inhibits the reaction.

The reaction appears to occur in the presence of tissue juice containing no organized cells. An oxidative, possibly phosphorylating, type of enzyme system appears to be involved. Muscle and prostate do not appear to metabolize testosterone significantly in this manner.

HYALINIZATION OF SEMINIFEROUS TUBULES AND CLUMPING OF LEYDIG CELLS: CHARACTERIZATION OF THE CLINICAL SYNDROME, ITS PHYSIOLOGY, DIAGNOSIS AND TREATMENT. *Heller, Carl G.* and *Warren O. Nelson.* Departments of Medicine and Anatomy, Wayne University College of Medicine, Detroit, Michigan.

A study of the clinical characteristics, hormonal excretion and testicular biopsies of twenty patients with hyalinization of the seminiferous tubules has led us to expand the limits of the syndrome recently described by Klinefelter, Reifenstein and Albright. In order to be admitted to the present category a patient must have the following: A) *Constant features* which are invariably present—1) small atrophic testes in which biopsy reveals hyalinization of the tubules and clumping of Leydig cells, 2) azoöspermia, 3) elevation of urinary gonadotrophins; B) On the basis of their *variable features* the patients are further classified as belonging to one of the following three subdivisions—1) eunuchoidal, 2) moderately eunuchoidal or 3) normal. These variable features include: a) skeleton which may or may not exhibit delay in osseous development and may show eunuchoidal or normal measurements; b) gynecomastia may be marked, mild or absent; c) external genitalia vary from completely infantile to entirely normal; d) the pattern of hair distribution may be prepuberal, puberal or normal adult in type; e) pitch of voice may be high or low; f) muscle development and strength vary from poor to normal; g) Leydig cells vary in quality; h) 17-ketosteroid excretion may vary from normal to very low; i) estrogens are not elevated, but may be normal or low. Physiology and therapy will be discussed.

HYALINIZATION OF SEMINIFEROUS TUBULES AND CLUMPING OF LEYDIG CELLS: MICROSCOPIC PICTURE IN THE TESTIS AND ASSOCIATED CHANGES IN THE BREAST. *Nelson, Warren O.* and *Carl G. Heller.* Departments of Anatomy and Medicine, Wayne University College of Medicine, Detroit, Michigan.

In the syndrome under consideration the testes are small (pea-size to almond-size). The seminiferous tubules are small and uniformly show hyalinization of the lamina propria. This is a progressive process and varies from a minor degree of sclerosis to complete hyalinization. Spermatogenesis is affected in proportion to the extent of the sclerotic process. In most testes Sertoli cells only are seen and in completely hyalinized tubules even these are absent. In all instances the interstitial cells of Leydig are clumped to a greater or lesser degree. These clumps frequently are so large as to suggest small adenomatous growths. The number and condition of the Leydig cells are variable and in agreement with the clinical picture and excretion of 17-ketosteroids. The more masculine patients have Leydig cells which are more numerous and which present a picture of activity, while the interstitial cells in less masculine patients usually are less numerous and present a picture of diminished function. The microscopic condition of the breasts varies from that usually encountered in normal males to one of hyperplasia of the parenchymatous elements and the interlobar stroma. The extent of the change corresponds in general with the presumed functional activity of the cells of Leydig.

SOME EFFECTS OF TESTOSTERONE, TESTOSTERONE PROPIONATE, METHYL TESTOSTERONE, STILBESTROL, AND X-RAY THERAPY IN A PATIENT WITH CUSHING'S

SYNDROME. *Martin L. Deakins, Harry B. Friedgood,* and *Joseph W. Ferrebee.* The Laboratory of Dental Medicine, Harvard School of Dental Medicine; the Department of Medicine, Harvard Medical School; and the Medical Clinic of the Peter Bent Brigham Hospital, Boston, Mass.

The metabolic and clinical effects of testosterone propionate, stilbestrol, methyl testosterone and testosterone were observed under controlled conditions in a girl, aged 15, with Cushing's syndrome. Injections of testosterone propionate, 25 mg. per day, caused a diminution in nitrogen excretion and a disappearance of creatinuria. Stilbestrol, 2 to 10 mg. per day, orally, did not affect nitrogen excretion but perhaps induced a moderate increase in creatine excretion and a decrease in creatinine excretion. Methyl testosterone, 40 mg. per day, orally, caused a striking decrease in nitrogen excretion and a tremendous increase in creatine excretion; creatinine excretion remained unaltered. Testosterone, 40 mg. per day, orally, did not produce unequivocal changes in excretion of nitrogen, creatine or creatinine.

Irregular increases in 17-ketosteroid excretion occurred during administration of testosterone propionate. Methyl testosterone caused an initial 3-day increase in 17-ketosteroids followed by a depression. Free testosterone caused a precipitous and marked rise in 17-ketosteroid excretion.

The androgens induced distressing accentuation of acne, hirsutism and hypertrophy of the clitoris. Stilbestrol improved these conditions but the benefit was not striking and was associated with certain toxic reactions and a transient hyperglycemia. X-ray therapy appeared to improve the subjective and objective manifestations of the disease.

CASE OF CUSHING'S SYNDROME TREATED WITH TESTOSTERONE PROPIONATE. *Whitelaw, M. J.* Charles S. Wilson Memorial Hospital, Johnson City, New York.

The case is presented of a boy, age 17, showing typical signs of Cushing's syndrome, osteoporosis of the spine and ribs, diabetic glucose tolerance curve, resistant to insulin, hypertension, decrease in libido, hirsutism, generalized muscular weakness and buffalo type of fat distribution and purple striae with normal genitalia. The 17-ketosteroids were normal. The serum sodium was markedly elevated. X-ray plates of the sella turcica and adrenal regions showed normal conditions. Under testosterone propionate treatment the boy showed marked gain in strength.

TITERS OF URINARY GONADOTROPINS IN OLD EUNUCHS WHO HAD BEEN CASTRATED PREPUBERTALLY AND EVIDENCE OF SUPRANORMAL SECRETORY ACTIVITY OF AN ENDOCRINE GLAND FOR FORTY TO FIFTY YEARS. *Hamilton, James B., Hubert E. Catchpole,* and *Charles C. Hawke.* Departments of Anatomy Yale University and University of Missouri Schools of Medicine, and the Adolescence Study Unit and Laboratory of Physiology, Yale University School of Medicine, New Haven, Connecticut.

Four men, who had been castrated between eight and twelve years of age and at the time of the present study had been eunuchs for 44 to 51 years, excreted large quantities of urinary gonadotropins—160, 160, 40 and between 40 and 80 M.U. daily. These values far exceeded the average daily excretion of 10–25 M.U. by ten normal men 55 to 76 years old. The presence of large amounts of urinary gonadotropins in prepubertally-castrated eunuchs seems to date from the normal chronological age of puberty, if not slightly earlier, as judged by collateral studies of two boys castrated before the advent of manifest puberty. If elevated titers of gonadotropins in castrate individuals reflect augmented secretion of gonadotropins by the pituitary gland, the continued production of greatly supranormal quantities of gonadotropins during most of the lifespan of these eunuchs provides a striking illustration of prolonged hyperfunction of an endocrine gland. Although primary gonadal insufficiency comparable to that in women past the menopause does occur

in old men, as after bilateral orchidectomy or destruction of the testes, climacteric-like conditions would not appear to be either a usual or a normal sequela of ageing in men.

THE USE OF THE SOMATOTROPIC PROPERTIES OF TESTOSTERONE PREPARATIONS IN CHRONIC DEBILITY.* *Reifenstein, Edward C., Jr., Anne P. Forbes, Lawrence W. Kinsell,* and *Fuller Albright.* From the Department of Medicine of the Harvard Medical School and from the Medical Service of the Massachusetts General Hospital, Boston, Massachusetts.

Testosterone compounds induce retention of nitrogen, sulfur, potassium, and phosphorus in the proportions that they exist in protoplasm. The evidence indicates that these substances possess protoplasmic anabolic (somatotropic) properties. Loss of protoplasm accompanies many disease processes: in most instances at the proper time during convalescence anabolism of protoplasm occurs spontaneously and restores the integrity of the tissues. Evidence suggests that these adaptations of the individual to a damaging event are under the control of the adrenal cortex; these phenomena have been discussed by Selye under "the alarm reaction." In chronic debility the phase of protoplasmic anabolism fails to occur; hence treatment with somatotropic agents seems indicated. The present investigations consist of nitrogen balances and other pertinent data before and after testosterone therapy in a patient with chronic debility (rheumatoid arthritis) and in a patient on an inadequate protein diet. In the former tissue anabolism was re-initiated; in the latter tissue catabolism for energy purposes was inhibited.

THE EFFECTS OF TESTOSTERONE, TESTOSTERONE PROPIONATE, AND PREGNENOLONE ON PROTEIN FABRICATION IN MAN. *Abels, Jules C., Nelson F. Young, Howard C. Taylor, Jr., C. P. Rhoads.* Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York City.

Ninety mg. of testosterone or 100 mg. of testosterone propionate were administered daily to 5 male subjects for from 7 to 40 days. The subjects were maintained on constant diets of known nitrogen content. The steroids administered induced nitrogen retention to account for from 200 to 392 gm. of protein. However, the total circulating albumin and globulin decreased in all instances. With further administration of the steroids, the circulating protein fractions eventually increased towards the pretherapy level. The plasma volume and extra cellular fluid were not significantly effected by either testosterone or its propionate. One hundred mg. of pregnenolone were later administered daily to one subject for 36 days. Although this steroid induced considerably less nitrogen retention than did the testosterone, the amount of circulating protein formed was considerably greater. No preliminary decrease of circulating protein occurred. The simultaneous increase in extracellular fluid and plasma volume was so great that the subject became edematous. It would appear from these studies, therefore, that the administration of testosterone or testosterone propionate primarily induced tissue protein fabrication even at the expense of circulating protein. In contrast, pregnenolone induced primarily circulating protein formation, but the associated extra-cellular water retention was too great to suggest clinical use for this material.

COMPARISON OF URINARY 17-KETOSTEROIDS OF TESTICULAR AND ADRENAL ORIGIN. *McCullagh, E. Perry* and *Wendell Bowman.* Cleveland Clinic, Cleveland, Ohio.

Colorimetric determinations for 17-ketosteroid and androgen bio-assays were done on a group of five male castrates and six male patients with Addison's disease

* These studies were supported by grants from the Josiah Macy, Jr. Foundation; and from the Committee on Endocrinology of the National Research Council. The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Massachusetts General Hospital.

The results were compared to determine what difference, if any, existed between the two groups. Steroids in the castrate should come from the adrenals, while those in the Addison's patient should be derived principally, if not wholly, from the gonads. Forty-eight hour specimens of urine were pooled, hydrolyzed, and extracted with butyl ether. One half of the butyl ether extract was assayed for androgens by the capon comb growth method. The other half was fractionated into ketonic, non-ketonic, and total neutral fractions. The average androgen level in the castrate group was 6.9 I.U., and in the Addison's group was 8.4 I.U. For the ketonic fractions 17-ketosteroids were 3.3 and 3.5 mg. per 24 hours respectively. The castrate group showed some correlation between the ketonic fractions and the androgen assays. The Addison's group did not. No measurable β-ketosteroids could be found in the pooled extracts of either group. As anticipated, similar determinations in women with Addison's disease showed a very low level of 17-ketosteroid excretion even in the total neutral fraction. Fractionation showed that practically all the color was due to nonketonic chromogens. It seems evident that the similarity of the assays in the castrate and Addison's groups makes it impossible to show any difference as judged by the data observed. However, since the majority in both groups were in fairly good health it seems reasonable that the sum of the average 17-ketosteroid values for the ketonic fractions of both groups could be taken as the lower limit of the normal 24-hour range. This would bring the values for the total crude extract to about 11 mg. and those for the ketonic fraction to about 7 mg. per 24 hours. Further observations regarding quantitative gonadotropins, sperm counts, and prostatic atrophy have been made in these cases.

METABOLIC STUDIES ON THE URINARY EXCRETION OF KETOSTEROIDS IN NORMAL INDIVIDUALS AND IN PATIENTS WITH ADRENAL DYSFUNCTION AND CANCER. *Dobriner, Konrad, C. P. Rhoads, Seymour Lieberman, B. R. Hill, Louis F. Fieser.* Memorial Hospital, New York City, and Converse Memorial Lab. Harvard University, Cambridge, Massachusetts.

The determination of the 17-ketosteroid levels in the urines of normal individuals and of patients does not allow for a distinction between the different kinds of steroids excreted nor does it permit the quantitative estimation of the amount of each compound present, both of which may be significant for certain disorders. Individual urine collections were made and the steroid content studied in a group of normal men and women of different ages, in pregnancy, in patients with adrenal dysfunction (adrenal hyperplasia, cortical tumor and Cushing's syndrome), and in patients with cancer and with leukemia. The neutral fractions obtained from these urines were separated into the ketonic and non-ketonic material. The ketonic components were further divided into their alpha and beta ketonic fractions. The non-ketonic material was separated into its alcoholic and nonalcoholic constituents. A total of 33 different ketosteroids were isolated by systematic quantitative chromatographic analysis of the alpha ketosteroids from individual urines. Seven of these have been previously described in the literature. Only 9 alpha ketosteroids are constantly found in the urine of normal men and women. Their distribution also also follows a consistent pattern. About 60 to 70 per cent of the alpha ketosteroid fraction consists of androsterone and its isomer, alpha hydroxyaetiocholanolone which were present in a ratio of about 1:1. The urine of pregnant women and of patients with adrenal hyperplasia and adrenal cortical tumors revealed not only the presence of a great number of ketosteroids not found in normal urine, but also a distinct difference in the pattern of their distribution. In the urines of the there cancer patients studied completely, ketosteroids were present which were not found in the urine of six normal persons. In others, androsterone was absent or an abnormal ratio of androsterone to aetiocholanolone was found; this ratio is similar to that in adrenal hyperplasia or pregnancy. The study of the alpha ketosteroid

patterns in normal and diseased individuals revealed marked differences in the distribution and types of compounds present. These results give more detailed information with regard to the disturbances of steroid metabolism.

THE URINARY 17-KETOSTEROIDS OF A PATIENT WITH AN INTERSTITIAL CELL TUMOR OF THE TESTES. *Hoffman, M. M.* McGill University Clinic, Royal Victoria Gospital, Montreal, Canada.

The urine of a man, 41 years of age, with a malignant tumor of the interstitial cells of the testis with extensive metastases was adjusted to pH 1 and extracted with butanol. The extract was submitted to simultaneous hydrolysis and extraction. The neutral fraction of the hydrolyzed material was separated into ketonic and non-ketonic substances. The ketonic substances were further fractioned into digitonin-precipitable and digitonin-non precipitable hydroxy-ketones and non-hydroxy-ketones. Each of these three fractions was submitted to repeated chromatographic analyses. Isolated and identified, with yields expresssed as milligrams per 24 hours, were androsterone (271.6) aetiocholanol-3 (α)-one-17 (62), Isoandrosterone (6.1), and Δ^2-androstenone-17 (38.1). Dehydroisoandrosterone could not be isolated. In addition to these, a number of unidentiqed compounds have been isolated. Androsterone accounts for most of the urinary 17-ketosteroids excreted by this patient, whereas in patients with carcinoma of the adrenal cortex dehydroisoandrosterone appears to be the major urinary 17-ketosteroid. The failure to isolate dehydroisoandrosterone in this case supports the hypothesis that this steroid is a metabolite of the steroid hormones of the adrenal cortex rather than of the testicle.

THE EXCRETION OF NEUTRAL URINARY STEROIDS IN STRESS. *Freeman, William, Gregory Pincus,* and *Everett D. Glover.* Research Service of the Worcester State Hospital and the Physiological Laboratories, Clark University, Worcester, Massachusetts.

Normal male subjects and mental patients have been subjected to the stresses of exposure to cold, pursuit-meter operation and psychological tests. The excretion of 17-ketosteroid during control and stress periods has been determined by the application of the Zimmerman and $SbCl_3$ reactions to the neutral ketonic fractions of urines collected during these periods. The normal subjects regularly exhibit an increased excretion of 17-ketosteroid during stress with a sharp post-stress decline. Responses among the patients are varied, but failure to show the stress rise is rather general, and a rise in 17-ketosteroid excretion in the post-stress period is marked. The excretion of atypical neutral steroid by the patients and of quantitatively abnormal β-ketosteroid is indicated.

THE EFFECT IN MAN OF CHRONIC INTERMITTENT ANOXIA ON GLUCOSE TOLERANCE AND THE URINARY EXCRETION OF SODIUM, POTASSIUM, CHLORIDE, PHOSPHORUS, NITROGEN AND 17-KETOSTEROIDS.* *Bryan, A. Hughes* and *Henry I. Ricketts.* Department of Medicine, School of Medicine, University of Chicago, Chicago, Illinois.

A considerable body of evidence has shown that exposure of anoxia profoundly affects the adrenal cortex of animals. The rapid development of aviation has made it desirable to determine whether anoxia produces in man the evidence of adrenal cortical stimulation observed in animals. To this end, 5 experiments were performed on 4 human subjects, 19 to 28 years of age, exposed without much physical activity to altitude pressures of 11,500, 16,600, and 18,000 feet from 4 to 6 hours

* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago.

daily, 6 days a week, for from 4 to 6 weeks. The subjects were on constant weighed diets and careful metabolic technic was followed throughout. The observations consisted of (1) measurements, in fractional and 24-hour urine specimens of sodium, potassium, chloride, phosphorus, nitrogen and 17-ketosteroids and (2) glucose tolerance. In some cases complete balance studies were done. With the possible exceptions of a slight increase in potassium excretion in two subjects a transient rise in 17-ketosteroid excretion in one subject and a moderate decrease in tolerance for glucose in two, the results obtained provide no evidence that the human adrenal cortex is influenced by chronic intermittent anoxia under the conditions of these experiments.

THE 17-KETOSTEROID EXCRETION IN DAMAGE AND CONVALESCENCE. *Stevenson, J. A. F., Victor Schenker,* and *J. S. L. Browne.* McGill University Clinic, Royal Victoria Hospital, and the Henry J. Elliott Research Laboratory, Shriner's Hospital, Montreal, Canada.

The daily excretion of total neutral 17-ketosteroids in the urine has been studied, using the Holtorf-Koch modification of the Zimmerman reaction, in some 30 males following various types of damage, (burn, fracture, operation, infection, hemorrhage) during convalescence, and in four healthy males. The majority were previously healthy, suffering acute damage, but some were chronically ill patients undergoing operation. In those followed from the onset of damage a sharp rise in 17-ketosteroid output (confirming Forbes) followed by an immediate fall was frequently but not invariably observed. There was then a gradual rise in daily excretion as convalescence progressed. During convalescence, marked peaks of 17-ketosteroid excretion were frequently seen, not only on further damage such as minor operations, infections, etc., but also on exertion, such as, first time in wheel chair, first walk, etc., which were not associated with changes in 17-ketosteroid in the later convalescence or in healthy active people. In two male children followed during damage, the 17-ketosteroid exretion remained relatively constant. The significance of these changes in 17-ketosteroid excretions and their possible relationship to adrenocortical function in damage, the differences in reaction of the healthy, the chronically ill, the child, and the convalescent are discussed.

PROBLEMS IN THE TREATMENT OF CARDIAC FAILURE IN MYXEDEMA. *McGavack, T. H.,* and *David Schwimmer.* New York Medical College, New York City.

The major problem confronting the clinician who must treat myxedema complicated by cardiac failure is the necessity for restoring the competency of the heart while increasing the load it must assume. Patients with hypothyroidism with demonstrable cardiac disease have been studied in states of thyroid insufficiency and cardiac failure. The influence of desiccated thyroid substance on cardiac compensation and thyroid insufficiency have been correlated with values for the basal metabolic rate, the circulation time, cholesterol and cholesterol esters of the blood, and capillary permeability. From these data, effort will be made to establish criteria by which therapy may be applied satisfactorily.

THE INFLUENCE OF THYROIDECTOMY ON THE PROMINENCE OF THE EYES IN THE GUINEA-PIG AND IN MAN. *Dobyns, B. M.* The Mayo Foundation, Rochester, Minnesota.

A new and more accurate method of measuring exophthalmos in guinea-pigs has been devised. This method was employed to determine the day-by-day changes in the prominence of the eyes of guinea-pigs after thyroidectomy. The experiment was controlled by a new application of skull measurement and by control experiments on normal animals. An increase in the prominence of the eyes after thyroidectomy was found. Histologic changes were studied in various tissues. Exophthalmometric measurements were obtained in 233 consecutive cases of

exophthalmic goiter and adenomatous goiter with or without hyperthyroidism. Measurements were made before and at intervals of one to four days after thyroidectomy. In all but nine cases some increase in the prominence of the eyes developed after thyroidectomy. This increase appeared to bear some relation to the basal metabolic rate. Less or no significance was found in its relation to the size of the thyroid, the amount of thyroid removed, the previous prominence of the eyes, the presence of thyroiditis and the sex. Control observations were made on patients subjected to other surgical procedures.

NON-PERMEABILITY OF THE MAMMARY GLAND TO SYNTHETIC THYROPROTEIN. *Turner, C. W.* and *E. P. Reineke.* University of Missouri, Columbia, Missouri.

Guinea pigs were given approximately 100 ml. of milk daily for two weeks from cows receiving massive doses of protamone (synthetic thyroprotein). In a second trial with guinea pigs, milk from cows receiving a moderate dose of protamone was fed for six weeks. Frequent determinations of metabolism during both experiments failed to show any significant differences between these animals and paired controls receiving a normal milk diet, or normal guinea pigs on a stock diet. The experimental group while still on the milk diet showed a 25 per cent increase in metabolism when 2.5 mcg. of l-thyroxine was injected daily for two weeks. In a similar experiment with thyroidectomized goats no metabolic stimulation or observable improvement of the cretinism could be detected as a result of feeding milk from protamone-fed cows for two weeks. These animals responded markedly, however, when $\frac{1}{4}$ gram of protamone daily was fed directly. No elevation of the basal metabolism could be detected when a quart of milk daily from protamone-fed cows was consumed by two human subjects. Significant elevation of the basal metabolic rate occurred when $\frac{1}{4}$ to 1 grain of protamone daily was given orally. It was concluded that the mammary gland does not permit passage of biologically detectable amounts of thyroidal hormone when protamone is fed to lactating cows.

IMMUNE REACTIONS IN RABBITS AND RATS AFTER INJECTIONS OF THYROID GLOBULIN. *Meyer, Arthur E., Constance M. Stickney, David Marine* and *Jacob Lerman.* From the Research Laboratories, The Maltine Company, the Laboratories Division, Montefiore Hospital, New York and the Thyroid Clinic of the Massachusetts General Hospital, Boston, Massachusetts.

Sterile solutions of globulin from human and pig thyroids were injected intraperitoneally in rabbits at suitable intervals. An initial rapid loss in weight was followed by slow gain. The increased metabolism, however, persisted on continued treatment for 10 weeks. The precipitin test in the serum became positive at a dilution of 1:10,000. The thyroids of the treated rabbits were small but produced metabolic stimulation when fed to thyroidectomized rats. The testes showed decreased spermatogenesis. Injection of the anti-thyroid serum into normal rabbits caused a drop in B.M.R. and prevented the response to injected thyroid globulin. Prolonged injections in rats inhibited the thyroids but produced no detectable antibodies. Sera from such rats did not prevent the full effect of thyroid globulin when injected into other rats. Spermatogenesis was not affected in the rat.

ON THE MECHANISM OF ALLOXAN DIABETES. *Goldner, Martin G.* and *George Gomori.* Department of Medicine, University of Chicago.

It has been shown in previous experiments by ourselves and others that a single intravenous injection of alloxan produces sustained diabetes mellitus in dogs, rats and rabbits. The anatomical basis of this diabetes is the degeneration of the islets of Langerhans, especially of the beta cells. The mechanism of alloxan diabetes has been further investigated. Alloxan-treated animals show glucose tolerance tests typical of diabetes mellitus; the diabetes improves on carbohydrate restriction

and on insulin treatment. Alloxan does not inhibit the action of insulin. Simultaneous treatment with insulin and alloxan prevents the development of hyperglycemia but does not protect against the necrosis of the islet cells. Pre-treatment with phloridzin does not prevent the development of alloxan diabetes. Adrenalectomy prevents the initial alloxan hyperglycemia, and the transitory alloxan hypoglycemia does not occur after pancreatectomy. Assays have proved that the pancreas of alloxan diabetic dogs is depleted of insulin. Several chemical compounds related to alloxan (e.g., alloxantin, dialuric acid, violuric acid and barbituric acid) were found ineffective. Alloxan in a buffered solution was also ineffective.

MODIFIED PROTAMINE ZINC INSULIN: COMPARISON WITH GLOBIN ZINC INSULIN AND WITH EXTEMPORANEOUS MIXTURES. *MacBryde, Cyril M.* and *Robert S. Reiss.* Department of Internal Medicine, Washington University School of Medicine, and the Barnes Hospital, St. Louis, Missouri.

In more than 100 diabetic patients modified protamine zinc insulin controlled the majority of uncomplicated cases with a single injection daily. Three-fourths of the insulin content acts slowly, while one-fourth acts rapidly. Of numerous extemporaneous mixtures, the one containing two parts crystalline to one part protamine zinc insulin seems most promising. Modified protamine zinc insulin was compared directly with globin zinc insulin and with the 2:1 mixture in a series of carefully controlled diabetic patients. Twenty-four hour blood sugar curves were obtained. It appeared that any of the intermediate insulins, as well as standard protamine zinc insulin, give fairly good results when the single dose is less than 40 units daily. Differences between the insulins become apparent with larger doses. Globin zinc insulin causes mid-afternoon hypoglycemia frequently. It also fails to control the rise in blood sugar after breakfast. The 2:1 mixture gives irregular results, with a tendency toward early morning hypoglycemia. The rise in the blood sugar after breakfast is partly controlled, but less well than with modified protamine zinc insulin. Modified protamine zinc insulin establishes better regulation than globin zinc insulin or the 2:1 mixture or other mixtures tried. With a single dose before breakfast the blood sugar can be kept within approximately normal limits during the night as well as during the day.

REVIEW OF 100 CASES OF DIABETIC NEUROPATHY FOLLOWED FROM ONE TO TEN YEARS.* *Rudy, Abraham* and *Samuel H. Epstein.* Medical and Neurologic Services of the Beth Israel Hospital, Boston, Massachusetts.

Diabetic neuropathy is a disseminated neurologic disturbance involving the entire nervous system, central as well as the peripheral. Only half the cases reviewed showed signs of peripheral neuropathy. It was most common between the ages of 50 and 70 years; least common below 40. Loss of weight preceded the neurologic symptoms in over half of the cases. The neuropathy occurred in acute diabetes before treatment was instituted and in some cases soon after control by diet alone or with insulin. It was observed in all types of diabetes mild and severe, controlled, semicontrolled and uncontrolled. Half of the cases were on a diet with insulin, when the neuropathy developed. In only 66 cases the diet was inadequate. Diabetic neuropathy is frequently accompanied by generalized manifestations of thiamine deficiency. In 41 cases signs of deficiency in various B vitamins were seen. In 90 per cent of the cases no vitamin therapy had been used before the neuropathy developed while 93 per cent received vitamin B in some form during the treatment of the neuropathy; 27 cases received yeast only and 45 cases were given parenteral thiamine chloride and some vitamin B-complex also. Marked improvement of the

* Aided by a grant from the Charlton Research Fund, Tufts College Medical School.

neuropathy was seen in 74 cases. Only 37 cases recovered in less than 6 months and 29 cases in about 6 to 12 months. Several were completely cured of subjective complaints and abnormal neurologic manifestations.

PLASMA PROTEIN STUDIES IN DIABETES MELLITUS BY TISELIUS ELECTROPHORESIS TECHNIC. *Lewis, Lena A., Robert W. Schneider,* and *E. Perry McCullagh.* From the Departments of Physiology and Endocrinology, Cleveland Clinic Foundation, Cleveland, Ohio.

Plasma proteins in diabetes mellitus have been extensively studied in the past, using chemical precipitation for estimating the albumin-globulin content. In hopes that further information might be obtained on changes in plasma-protein fractions in various stages of the disease, electrophoretic studies were undertaken by Longworth's method with phosphate buffer pH 7.8. Total nitrogen was determined by Pregl's modification of the micro-Kjeldahl method. In uncomplicated diabetes showing extreme acidosis, total protein was normal but albumin greatly decreased. Most of the increase in globulin occurred in the β-fraction; α- and γ-globulin and fibrinogen remained normal. After diabetic control and months of adequate nutrition the protein picture was entirely normal. In a case of diabetic acidosis complicated by Staphylococcus aureus infection a marked increase in α- and β-globulin occurred. In many infections a high α-globulin with no increase in β-globulin is observed. In mild uncomplicated diabetes with very slight or no acidosis, the total protein was low but the relative percentage of albumin, α-, β-, and γ-globulin and fibrinogen was normal. After control of the diabetes the protein level rose to normal. Studies on diabetics showing retinal changes when the diabetes *per se* was well controlled showed low albumin and decreased or low normal total protein. Preliminary studies following a very high protein diet indicated some increase in albumin.

A STRESS-SURVIVAL TEST FOR CORTIN.* *Elmadjian, Fred* and *Gregory Pincus.* From the Physiological Laboratories, Clark University, Worcester, Massachusetts.

Adrenalectomized 40 to 60 gram rats tied down to a wire grid by all four legs for one hour exhibit symptoms of shock and die within four hours. The administration of desoxycorticosterone acetate, adrenal cortex extract, and certain 11-oxygenated corticosteroids prolongs survival. Typical dosage-response curves may be obtained with a variety of corticoid substances. The usefulness of this method as a quantitative assay for cortin will be discussed.

CORTIN-LIKE MATERIAL IN HUMAN AND MONKEY URINE. *Dorfman, Ralph I., Reginald A. Shipley, Benjamin M. Horwitt,* and *William E. Abbott.* Brush Foundation and Departments of Biochemistry, Medicine and Surgery, Western Reserve University, School of Medicine and Lakeside Hospital, Cleveland, Ohio.

Cortin-like material previously demonstrated in normal men's urine has been shown to be present also in the urine of normal women and of monkeys. Evidence is presented which indicates that this material arises in the adrenal gland and that the urinary concentration of cortin-like material may well be an index of adrenal cortical activity. Urinary cortin-like material was assayed by means of a cold protection test using immature adrenalectomized rats. Significance of difference between mean survival time of extract-treated and control rats was calculated. Urinary cortin-like material has been studied in intact, gonadectomized, adrenalectomized and gonadectomized-adrenalectomized monkeys—nine in all. Cortin-like material was found in 11 of the 21 urines from intact monkeys and in 4 of 9 urines from gonadectomized monkeys. This material was not found in any of 6 urines

* Aided by a grant from G. D. Searle and Company.

collected after adrenalectomy. In 6 experiments the administration of adrenal cortical extracts to adrenalectomized monkeys invariably was followed by the reappearance of cortin-like material in the urine. In 1 of 3 experiments, urinary cortin-like material was demonstrable after the administration of desoxycorticosterone to adrenalectomized monkeys. Urinary cortin-like material was studied in 9 normal men, 8 normal women and in 7 patients with Addison's disease. The urines of all men and women studied contained cortin-like material while only once in 15 assays of Addison's-disease urine was it possible to demonstrate active material. In 3 of 5 experiments in which adrenal cortical extracts were administered to Addison's-disease patients, significant amounts of active material were found in the urine. Studies on ethylene dichloride or chloroform extracts of normal men's urine disclosed that the active material is in the ketonic neutral fraction. At least a portion of the active material in urine appears to be present in a combined form.

CORTIN-LIKE SUBSTANCES IN URINE. *Venning, Eleanor H., M. M. Hoffman,* and *J. S. L. Browne.* McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

A method, based on solvent partition, is described for the extraction and purification of cortin-like substances in urine. This method permits a 25- to 100-fold concentration of the biologically active substances with a loss of approximately 25 per cent of the activity present in the crude urinary extracts. Extracts from postoperative urine are capable of maintaining the adrenalectomized rat, of protecting it against the lethal action of cold, and of causing glycogen deposition in the liver of the fasted adrenalectomized rats. Urine excreted after operation was found to contain three to 30 times the amount of cortin-like substances excreted by normal men. The active substances are ketones. Highly purified ketonic fractions contain 25 to 100 cold units per milligram (one cold unit represents the activity of ten gamma of corticosterone in the modified Selye-Schenker cold exposure test). The mouse has been used as a test animal for assaying the liver glycogen deposition effect of individual 24- or 48-hour specimens of urine. The excretion of these substances has been followed in normal males and females following muscular exercise and damage.

STUDY OF THE "ALARM REACTION" IN BURNED HUMAN SUBJECTS AND ITS POSSIBLE SIGNIFICANCE IN WAR INJURIES.* *Glendening, Mary Beth, Helen A. Winter, H. H. Williams, W. E. Abbott, J. W. Hirshfeld,* and *Carl G. Heller.* Departments of Medicine and Surgery, Wayne University College of Medicine, the Medical and Surgical Services of the City of Detroit Receiving Hospital and the Research Laboratories of the Children's Fund of Michigan.

This study is designed to correlate changes in nitrogen balance in burned patients with change in endocrine function which might accompany the "alarm reaction" in human beings. The excretion of estrogens, gonadotrophins, 17-ketosteroids, cortins and nitrogen intake and output have been followed for 1 to 7 five-day metabolic periods, beginning immediately after the burn was sustained. Estrogenic excretion tended to be higher during the first 10 days than subsequently, but remained within the limits of normal variation except that an abnormally high level was encountered in the most severely burned male patient. The excretion of gonadotrophic hormone remained within normal limits during the first 20 days following the burn. Subsequently increases were noted. In some cases the levels returned to normal. However, in one patient the increased levels of excretion were still observed one month after recovery. The 17-ketosteroid excretion fluctuated

* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Wayne University College of Medicine.

within the range of normal values. The concomitant occurrence of negative nitrogen balance and elevation of cortin excretion taken together with the changes in excretion of other hormones may elucidate the physiology involved in nitrogen retention and the repair of tissue and may suggest therapy for burned and wounded servicemen.

THE ROLE OF THE ADRENALS AND HYPOPHYSIS IN THE CONTROL OF SYSTOLIC BLOOD PRESSURE IN THE RAT. *Leathem, J. H.* and *V. A. Drill.* Department of Zoology, Rutgers University, New Brunswick, and the Department of Biology, Princeton University, Princeton, New Jersey.

Systolic blood pressure of the rat was studied in unanesthetized adult males following adrenalectomy, hypophysectomy and after administration of adrenal hormones. Normal blood pressures ranged between 110 and 120 mm. Hg. Adrenalectomy caused a sharp decrease in blood pressure which averaged 60 mm. six days postoperatively. A slight rise may occur followed by a fall in pressure below 60 mm. and death of the animal. Hypophysectomy is followed by a decrease in pressure to 80 mm. However this subnormal pressure is maintained for as long as seven months. A daily dosage of desoxycorticosterone acetate (0.5 mg.) that will maintain blood pressure at essentially normal levels in adrenalectomized rats for 20 days failed to prevent the drop in pressure following hypophysectomy. This dosage of D.C.A. seldom caused a mild hypertension in normal rats. Large doses of adrenal cortical extract, however, partially prevented the decrease in blood pressure following hypophysectomy. Average pressure 15 days postoperatively in treated rats was 95 mm. as compared with 83 mm. for operated controls and 111 mm. for the normal rat. It would appear that some factor in addition to the loss of cortical hormone is responsible for the lowering of systolic blood pressure after hypophysectomy in rats.

ESTROGEN FROM THE ADRENAL GLANDS. *Schiller, Joseph.* The Physiological Laboratory, Boston University, Boston and the Physiological Laboratories, Clark University, Worcester, Massachusetts.

Two mgm. per day of a highly purified adrenocorticotropic hormone preparation were administered by injection twice daily to several groups of adult female rats (7–10 animals per group). Daily variations in the vaginal smear were observed. No change was observed in normal diestrus females, but animals injected during estrus exhibited prolonged cornification. Ovariectomized and ovariectomized-adrenalectomized rats exhibited no vaginal smear changes. Proestrus smears were induced in ovariectomized partially hepatectomized rats. The proestrus smears resulting from partial hepatectomy in normal females were converted to fully cornified smears. Production of estrogen from the adernal cortex by corticotrophin stimulation is indicated. The adrenal glands of 8 mature female rats were grafted into the spleen. The estrous cycle was unaffected. All tests of adrenal cortex function such as life maintenance, cold resistance, liver glycogen and blood sugar levels indicated normality.

Papers Read by Title

THE INFLUENCE OF ADMINISTERED HORMONES IN PITUITARY INFANTALISM: CASE REPORT. *McGavack, T. H.* and *Kurt Lange.* New York Medical College, New York City.

A 49-year-old male with infantilism due to craniopharyngioma was studied. The influence of testosterone propionate, desoxycorticosterone acetate, α-estradiol dipropionate, and several pituitary extracts upon his generally depressed metabolic status was studied in relation to his general sense of well being and various laboratory findings. These included blood chemical findings (cholesterol and esters,

intravenous glucose-tolerance tests, and sodium and potassium); urinary excretion of creatine, sodium, potassium, and chloride; inulin clearance; capillary permeability; and response to ingestion of water as determined by the Kepler-Robinson test.

TREATMENT OF PITUITARY DWARFISM WITH CHORIONIC GONADOTROPIN AND MALE SEX HORMONE: END RESULTS. *Thompson, Willard O., Norris J. Heckel,* and *Phebe K. Thompson,* University of Chicago, Chicago, Illinois.

We have previously shown that in young boys chorionic gonadotropin and male sex hormone will cause rapid acceleration of growth similar to that which occurs normally at the time of puberty without treatment. The effect of these materials has now been observed in pituitary dwarfs over a period of several years. In this condition they also cause rapid growth, which involves not only the skeleton but also every tissue of the body, particularly the musculature. In the course of from 2 to 5 years the rate of growth decreases as epiphyseal closure sets in. The maximum increase in height observed was about 8 inches. The amount of growth appeared to be greater when treatment was started at the age of 9 to 10 years than when it was started at the age of 15 to 16 years. When the dwarfism was marked it was impossible to cause the skeleton to achieve normal height with these materials. The following conclusions would appear to be warranted. Male sex hormone will cause rapid acceleration of growth in pituitary dwarfs before the age of epiphyseal closure. This acceleration of growth takes place in the absence of the pituitary growth factor and is probably the result of a direct influence of male sex hormone on the growth of bone and other tissues. Male sex hormone is not a substitute for the pituitary growth factor or thyroid hormone in stimulating growth. It appears to influence primarily the component of growth which takes place at the time of puberty, namely, skeletal moulding. The administration of chorionic gonadotropin or male sex hormone is desirable in pituitary dwarfism. Treatment should be started at the age of 10 to 12 years and continued until epiphyseal closure sets in. Only in this manner is it possible to insure that the growth associated with puberty will take place.

THE IN VITRO METABOLISM OF TESTOSTERONE BY RABBIT LIVER. *Clark, L. C., Jr.* and *C. D. Kochakian.* Department of Vital Economics, University of Rochester, Rochester, New York.

Rabbit liver slices were incubated with a suspension of testosterone in serum. The resultant mixture was analyzed by extracting with fat solvents and fractionating by chemical and physical means. The hydroxyketonic fraction yielded unchanged testosterone and very small amounts of cis-testosterone and a digitonin precipitable compound. The non-hydroxy-ketonic fraction consisted mainly of Δ^4-androstenedione-3,17. The non-ketonic fraction yielded a small amount of an unidentified 17-hydroxy compound. Some evidence was obtained for the existence of a conjugated testosterone.

THE INFLUENCE OF TESTOSTERONE PROPIONATE, METHYL TESTOSTERONE AND METHYL ANDROSTANEDIOL ON CREATINE METABOLISM. *Keutmann E. Henry, Samuel H. Bassett* and *Charles D. Kochakian,* Rochester, New York.

One eunuchoid, one undernourished and two normal young men were studied. Each had a constant small creatinuria probably caused by a constant amount of meat in the same daily diet.

Testosterone propionate caused a decrease of creatinuria. After withdrawal there was a rebound with a peak value about three times the amount in the control periods. Both the retention and the rebound were greater in the eunuchoid than normal persons. The excess creatine excreted during the rebound was more than the excess retained. This suggests that testosterone propionate accelerated the synthesis of creatine. Increased creatinine excretion supports this also.

Methyl testosterone and methyl androstanediol caused a decrease of creatinuria for a few days followed by a gradual increase to levels far above the control periods. With methyl androstanediol this continued for some time after withdrawal then decreased. When methyl testosterone was withdrawn there was a marked rebound from release of creatine which had previously been stored. Steady increase of creatinine excretion was observed while both compounds were given. This must mean that increased creatine was deposited.

A Study of the Intermediary Metabolism of Certain Adrenal Steroids.* *Pearlman, W. H.* and *Gregory Pincus.* Physiological Laboratories, Clark University, Worcester, Massachusetts.

Considerable indirect evidence supports the theory that the urinary 17-ketosteroids are in part derived from the adrenals but direct evidence is lacking to show that the numerous C-21 steroids elaborated by the adrenals are transformed in the animal body to 17-ketosteroids. The guinea pig is a suitable test animal for the study of 17-ketosteroid excretion following the administration of representative C-21 adrenal steroids for the following reasons: 1. Its small size allows the use of small amounts of the precious test compounds. 2. The guinea pig has been shown (Dorfman et al.) to be capable of excreting 17-ketosteroids following testosterone administration and to serve as a satisfactory subject for steroid metabolism studies. The 17-ketosteroid determinations were made on the neutral ketonic fraction of the urine extracts with the aid of the Zimmerman color test and also the antimony trichloride test of Pincus; the latter test appears to be more specific for 17-ketosteroids, especially for those closely related to androsterone. Current evidence indicates that typical urinary 17-ketosteroids may be derived from certain C-21 adrenal steroids.

Estimation of the Non-Ketonic Alcoholic Steroids in the Urine of Normal and Diseased Individuals. *Lieberman, Seymour* and *Louis F. Fieser.* Converse Memorial Lab. Harvard University, Cambridge, Massachusetts. *Konrad Dobriner,* and *C. P. Rhoads,* Memorial Hospital, New York City.

The application of the Zimmerman reaction for the quantitative determination of the 17-ketosteroids in the urine of normal and diseased individuals has yielded valuable clinical information. There has been no clinical method available for the quantitative estimation of the total non-ketonic alcoholic steroids. However, various investigators have demonstrated the presence of such compounds—especially pregnandiol. A method has been developed for the separation and quantitative estimation of the urinary non-ketonic alcoholic steroids. The method has been based on the formation of the phthalic or succinic half-esters of the alcohols and the subsequent electrometric titration of the resulting half-esters. The amounts of alcohol reacting are directly related to the acidity. The alcoholic steroids may be regenerated by the use of mild hydrolysis from the half-esters and the amount of steroid determined by weight or titration. The application of this technique to pure alcoholic steroids has resulted in the recovery of 90 to 100 per cent of the original material by the titration method and 95 to 105 per cent by the weight method. The application of these methods to the non-ketonic fractions obtained from human urines has given the following results. Normal men and women excrete 3 to 12 mg. per 24 hours. This increases to 30 to 50 mg. in cases of adrenal hyperplasia. In cases of tumors of the adrenal cortex, values of 27 to 83 mg. were obtained. In pregnancy the values were 24 to 27 mg. After the injection of 100 mg. of testosterone, only a slight increase of the non-ketonic alcohols was observed which is in contrast to the definite increase of the 17-ketosteroids. The main portion

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of the administered testosterone which does not appear as a ketonic metabolite also does not appear in the non-ketonic alcoholic fraction.

A Comparison of the Renotrophic with the Androgenic Properties of Steroids. *Kochakian, Charles D.* University of Rochester Medical School, Rochester, New York.

Forty steroids were studied for their renotrophic, androgenic and thymus-regressing properties by implanting pellets (14 ± mg.) of the compounds subcutaneously into castrated mice and weighing the organs after 30 days of treatment.

There was no parallelism between the renotrophic and androgenic properties. The renotrophic-to-androgenic ratio of the compounds demonstrating marked potencies was as follows: 17-Methyl androstanediol-3α,17α, (1.70); androstanediol-3α,17α (1.48); 17-vinyl testosterone (1.14); 17-ethyl testosterone (1.09); testosterone (0.90); testosterone propionate (0.88); 17-methyl testosterone (0.85); androstanol-17α, one-3 (0.79); normal mice (0.74); androstenedione-3,17 (0.63); testosterone-acetate-3, propionate-17 (0.56). The more potent steroids also were studied in a 10 day period of treatment. The renotrophic-to-androgenic ratio was higher than in the 30 day experiments for all of the steroids but the sequence for the various steroids remained the same.

Is Testosterone Therapy Indicated in Female Patients with Addison's Disease?* *Forbes, Anne P., Edward C. Reifenstein, Jr., Lawrence W. Kinsell* and *Fuller Albright.* From the Department of Medicine of the Harvard Medical School and from the Medical Service of the Massachusetts General Hospital, Boston, Massachusetts.

It is our belief that the androgens arising from the adrenal cortex have an important somatotropic function (anabolism of protoplasm). In Addison's disease all of the hormones of the adrenal are deficient; the loss of the adrenal androgenic steroids reduces the anabolism of protoplasm. This loss is of serious consequence in females since in them the adrenal constitutes the sole source of androgen. Therefore testosterone preparations with their somatotropic properties seem indicated in women with Addison's disease. Complete metabolic studies of nitrogen, phosphorus and calcium in a female patient with Addison's disease before and after testosterone treatment are presented.

Cortin Excretion in Burned Patients.† *Winter, Helen A., Mary Beth Glendening, H. H. Williams, W. E. Abbott, J. W. Hirshfeld* (all by invitation) and *Carl G. Heller.* Departments of Medicine and Surgery, Wayne University College of Medicine, the Medical and Surgical Services of the City of Detroit Receiving Hospital and the Research Laboratories of the Children's Fund of Michigan, Detroit, Michigan.

Urinary cortin excretion has been determined immediately after burns had been sustained in 14 hospitalized patients and repeated determinations were performed at intervals during convalescence. The purpose was to correlate the excretion of cortin, the "S" or sugar hormones of the adrenal cortex, with the nitrogen balance and excretion of other hormones in the burned patients. Twenty-four hour aliquots of urine from a pool collected over a five day metabolic period were ex-

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† The work described in this paper was done under a contract, recommended by the Committee of Medical Research, between the Office of Scientific Research and Development and the Wayne University College of Medicine.

tracted with ethylene dichloride. Nine to ten adrenalectomized rats were given the extracted material orally on each dosage level, and the same number of non-treated adrenalectomized control rats were used for each assay. The survival of the rats subjected to a temperature of 1° C. is used as the index of activity. Results are expressed as "cold units" per 24 hours of urine as defined by Browne. Ten normal control patients excreted 5, 4 or less than 5 or 4 "cold units" per 24 hours. During the first metabolic period 11 patients excreted 20, 30 or more than 20 or 30 "cold units" per 24 hours. The three patients excreting less than 20 "cold units" included a 5-year-old child, an adult woman, and an adult male treated with growth hormone. The 11 patients with elevated cortin excretion continued to excrete large amounts in subsequent periods but this tended to be somewhat lower than in the first period. It is noteworthy that patients with small burns (as little as 8 per cent of the body surface burned) may excrete amounts of cortin as high as encountered in the more severely burned patients. Data on additional patients will be available at the time of presentation.

THE HORMONAL CONTROL OF THE URINARY EXCRETION OF CITRIC ACID. *Shorr, Ephraim, Alice R. Bernheim* and *H. Taussky*. The Russell Sage Institute of Pathology, The Departments of Medicine and Surgery, Cornell University Medical College and the New York Hospital, New York City.

Urinary excretion of citric acid is specifically influenced by steroidal hormones. The excretion curve during the menstrual cycle takes the following pattern: it is lowest at menstruation, rises to a midmenstrual peak approximately at ovulation, is sustained around this higher level until just before the next flow, then falls, generally abruptly to the low menstrual levels. Analysis of this phenomenon by administration of physiological amounts of estrogen, progesterone and testosterone to amenorrheic and post-menopausal subjects showed that: (1) estrogens significantly increase citric acid excretion; (2) progesterone given alone is without effect, but given immediately after estrogen partially sustains the high level previously induced by estrogen; (3) testosterone significantly reduces citric acid excretion. The cyclic variations in urinary citric acid during the menstrual cycle are interpreted as follows: (1) the initial rise during the first half of the cycle is due to increasing elaboration of estrogen by the growing follicle which is maximal at ovulation; (2) the persistence of the higher level during the luteal phase is due to continued estrogen elaboration at lower levels, synergized by progesterone; (3) the premenstrual drop and lower menstrual level are due to sharply reduced elaboration of both hormones, plus the influence of androgens. The significance of this hormonal function is under investigation.

SEXUAL CYCLE AND DIMORPHIC PIGMENTATION IN THE GOLDEN HAMSTER (CRICETUS AURATUS WATERHOUSE). *Kupperman, Herbert S.* (Introduced by: Robert B. Greenblatt). Department of Experimental Medicine, University of Georgia School of Medicine, Augusta, Georgia.

The reproductive cycle of the golden hamster was studied for a period of 18 consecutive months. Cyclic changes in the vagina, as observed by vaginal lavage, occur regularly at four-day intervals throughout the entire year. The proestrous smear in contradistinction to that observed in the rat is composed wholly of cornified cells. Estrus is designated by a thick, white, sticky smear composed predominately of nucleated cells with some cornified cells. This stage is associated in the early phases with receptivity of the female to the male, and somewhat later with post-ovulatory changes in the ovary. The metaestrous smear is characterized by a heavy infiltration of leucocytes associated with a few nucleated and cornified cells. The diestrous smear is indicated by the sparse number of leucocytic and cornified cells present in the smear. Vaginal introitus, although established as early as the 10th–15th day of life, is not indicative of ovarian stimulation. Cyclic changes in

the vagina with corpora lutea formation do not occur until the 33rd–38th day of life and continue, excluding pregnancy, until senility which is observed in animals 500–600 days of age. In the male, spermatogenesis commences on the 35th day of life and continues throughout the year, regardless of season until senility which, as in the female may be observed in animals 500–600 days of age. During the period of testicular activity, a pigmented area of 5–7 mm. in diameter is present on the dorsolateral subcostal region of the skin. The hair in this particular area is coarser than that seen in the rest of the pellage. The pigmented area is either absent or present only to a slight degree, with no difference in hair texture, in the female or castrated male hamster. Pellets of testosterone propionate, implanted subcutaneously, accentuate the degree of pigmentation in the normal male and induce its formation in the castrated male or female or normal female hamster. Pellets of α-estradiol dipropionate caused complete regression of the pigmentation area in the male hamster. The influence of the androgens is indicated further by the fact that the pigmented area, absent in males at birth, appeared with the initiation of testicular function and regressed in senile hamsters.

THE ACTIVITY OF URINARY EXTRACTS ON GLYCOGEN DEPOSITION IN LIVER AND AN IMPROVED METHOD OF ASSAY. *Eggleston, Nancy M., Konrad Dobriner* and *C. P. Rhoads.* Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York City.

The neutral ether-soluble fraction obtained from unhydrolyzed urine of normal and diseased individuals was studied to ascertain whether or not glycogen was deposited or maintained in the livers of adrenalectomized animals following administration of these fractions. The Reinecke-Kendall test using rats was found to detect the presence of 1 rat unit, and with mice, 0.5 rat unit could be determined. When the mice were not fasted prior to the first injection, the sensitivity of the test was increased so that 0.05 rat unit was effective, permitting the performance of a complete assay on a 36- to 48-hour specimen of urine. The technics applied will be described. There was activity in the crude neutral, ketonic and non-ketonic fractions; this finding confirms and extends the observations of Weil and Browne and Venning Hoffman and Browne and of Dorfmann et al. The daily urinary excretion in normal individuals was found to be between 0.5 and 2 rat units. The results of these tests on normal individuals and on patients with cancer and with adrenal cortical syndrome will be discussed.

THE EFFECT OF VITAMIN DEFICIENCIES ON ESTRADIOL INACTIVATION BY LIVER SLICES. *Singher, H. O., Howard C. Taylor, Jr., C. P. Rhoads* and *K. Unna.* Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York City.

Biskind and coworkers have demonstrated an interrelationship between B-complex deficiency and the production of experimental estrus by estradiol, in animals. They also have shown a connection between B-complex therapy and certain gynecological conditions in man. The present paper, in an attempt to find an approach to the enzyme systems involved in estradiol inactivation, deals with the effect of deficiencies of individual known members of the B-complex and vitamin A on the ability of rat and mouse liver slices to inactivate estradiol. Liver slices from riboflavin and thiamin deficient rats are unable to inactivate estradiol under conditions in which controls on the same diet but receiving adequate amounts of these vitamins possess this activity. The ability of liver slices from riboflavin and thiamin deficient animals to inactivate estradiol is a function of the tissue concentration of these vitamins. When rat liver riboflavin values fall below 12γ/gm. wet weight, the slices can no longer inactivate estradiol under experimental conditions. Similarly, when rat liver thiamin values fall below 1γ/gm., the inactivating ability is lost. When riboflavin or thiamin levels are brought back to normal, the reappear-

ance of the inactivating ability follows the rise in concentration of these vitamins. Pyridoxine, pantothenic acid, biotin and vitamin A deficiencies have no effect on estradiol inactivation under the same conditions.

THE EFFECT OF CASTRATION AND VARIOUS STEROIDS ON THE ARGINASE ACTIVITY OF THE TISSUE OF THE MOUSE. *Kochakian, Charles D.*, University of Rochester Medical School, Rochester, New York.

Mice castrated at 16.5–19.5 gm. body weight were implanted subcutaneously one month later with 14 ±1 mg. pellets of various steroids. Arginase determinations were made 10 and 30 days later. None of the steroids affected the enzyme content of the liver or intestine, but that of the kidneys was markedly increased by many of the steroids and in three instances decreased. The order of change in per cent difference per gram of tissue for the 30-day experiments was: Methyl testosterone, 632; Testosterone, 584; Testosterone propionate, 308; 17-Methyl androstanediol-3α, 17α, 269; Androstanol-17α, one-3, 135; α-estradiol, 88; Androstanediol-3α, 17α, 71; 17-Vinyl testosterone, 55; Testosterone-acetate-3, propionate-17, 35. Eighteen other compounds had no effect and isoandrosterone, 17-methyl androstanediol-3β, 17α and 17-methyl androstanediol-3β, 17α caused decreases of 33 to 39 per cent. The changes were not related to changes in kidney weight or amount of material absorbed. The increases in arginase activity represented greater amounts of enzyme and not a production of arginase activators. The kidneys of the castrated mice contained the same amount of total arginase but greater amounts per gram of tissue than those of the normal mice.

NEUTRAL NON-KETONIC URINARY CHROMOGEN IN THE $SbCl_3$ REACTION.* *Pincus, Gregory.* From the Physiological Laboratories, Clark University, Worcester, Massachusetts.

The non-ketonic, neutral fraction of the ether soluble extract of acid-hydrolized human and guinea pig urines contains material which reacts with $SbCl_3$ to give a typical blue color. The chromogen is excreted by human subjects in minimum amount at night and there is a diurnal rhythm of output markedly similar to that described for the urinary 17-ketosteroids. The amount of chromogen increases markedly in guinea-pig urines after the injection of certain androgenic and corticoid steroids. The probable steroid nature of the urinary chromogen will be indicated, and its role as an indicator of phases of steroid metabolism discussed.

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